Supplementary Information

Characterization of rare germline variants in familial multiple myeloma

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Materials and methods

Ethical approval

Collection of patient samples and associated clinico-pathological information was undertaken with written informed consent and relevant ethical review board approval at respective study centers in accordance with the tenets of the Declaration of Helsinki. The study was approved by the ethics committee of the Medical Faculty of the University of Heidelberg (study number S-589/2016), the Lund University Ethics Review Board (dnr 2010/131 and 2013/54) and the ethics committee of the Ärztekammer des Saarlandes (#154/08, #177/08). All patients enrolled by the University Medical Center Groningen (UMCG) were part of the Groningen-Heidelberg-Stettin EU TRANSCAN Familial cancer whole genome sequencing project. They were referred to UMCG clinically for diagnostics and counselling because of their cancer family history. Enrollment was therefore regarded as being directly in line with the clinical reason for testing and not subject to review by Ethics review board of the UMCG. All UMCG participants did sign informed consents stating that they agreed to undergo whole genome sequencing in this project to identify the cause of cancer predisposition (if any) in their families.

Multiple myeloma families

Altogether, 21 families with 46 affected and 20 unaffected family members were recruited (Supplementary Figure 1). Fifteen of the families were recruited in Germany, 12 in Heidelberg (1), two in Homburg and one in Ulm. Four families came from Sweden (2) and two from the Netherlands. Each family had at least two individuals diagnosed with MM or its precursors MGUS and smoldering MM (SMM). Also, patients with solitary plasmacytoma and AL amyloidosis were enrolled. Participating unaffected family members recruited in Heidelberg were analyzed for the
following parameters: blood count, creatinine, and glomerular filtration rate, calcium,
immunoglobulin levels, free light chains and their ratio, protein electrophoresis, and
immunofixation in serum and urine in order to exclude undetected MM or its precursor stages (1).
Only individuals with negative immunofixation in serum and urine were considered as unaffected.
The pedigrees of the German and Dutch families are shown in Supplementary Figure 1, each of
the four Swedish families consisted of two first- or second-degree relatives diagnosed with MM.

Whole Genome Sequencing

Samples from Heidelberg and the Netherlands were whole-genome sequenced at the core facility of
DKFZ. WGS of the MM family members was carried out using the Illumina X10 platform on DNA
isolated from the peripheral blood samples (QIAamp® DNA Mini Kit. Qiagen). WGS was
performed as paired-end sequencing with a read length of 150 bp. BWA mem (version 0.7.15, with
parameters: -T 0) and Sambamba (version 0.6.5, with parameters: t 1 -l 0 --hash-table-
size=2000000 --overflow-list-size=1000000 --io-buffer-size=64) were used to map sequences to the
reference human genome (build GRC37, assembly hs37d5) and to remove duplicates, respectively.
Platypus (version 0.8.1) was used for variant calling of small variants, single nucleotide variants
(SNVs) and indels.

Whole-exome sequencing

Samples from Homburg, Ulm and Sweden were whole-exome sequenced as described in
Halvarsson et. al. (2). Following isolation of exonic DNA by hybrid capture (SureSelect; Agilent,
Santa Clara, CA), libraries were constructed using standard methods, and sequenced on Illumina
HiSeq instruments to target mean coverage of 100x. Reads were aligned to GRCh37 and the
resulting SAM files were converted to BAM files using Picard (http://picard.sourceforge.net). The
variants were called using multi sample processing mode of the Unified Genotyper tool from GATK. To calculate background frequencies, we used pre-existing WES data from the NHLBI GO Exome Sequencing Project, which were subjected to variant calling in the same run (3). Genotypes with quality <10 or read depth <8 were marked as missing data. Variants with >10% missing data were excluded, as were samples with >5% missing data. A total of 3,597 controls of European ancestry were selected from the ESP data using the first two principal components calculated using –mds-plot option in PLINK (4).

**Variant annotation and filtering**

The processed list of WES variants and raw WGS variants were analyzed together in the following downstream steps. The variants were annotated with Gencode v19 gene definitions using ANNOVAR (5) and further with dbSNP (6), 1000 Genomes phase III (7), dbNSFP v2.9 (8), and ExAC (9) read depth >10. Minor allele frequency (MAF) of 0.1% was used with respect to 1000 Genomes phase III and non-TCGA exome aggregation (ExAC, version 0.3) data to remove common variants, and variant frequency of 2% from the local data sets was used to remove technical artefacts. In order to control for family relatedness and sample swaps, a pairwise comparison of variants among the cohort was carried out.

**Variant prioritization: missense variants**

Variant prioritization was performed using our in-house developed pedigree-based pipeline, Familial Cancer Variant Prioritization Pipeline (FCVPP) version 2 (10). Pedigree segregation was the first criterion the variants were screened for. Family members diagnosed with MM, MGUS or AL amyloidosis were considered as cases, also members with plasma cell dyscrasia, solitary plasmacytoma and aberrant plasma cell clone were considered as variant carriers and unaffected
members as non-carriers, unless they were more than 10 years younger than the earliest age of
diagnosis of cases in the family. After the pedigree segregation filtering, all the variants ranking
within the top 1% of potentially deleterious variants in the human genome were selected using the
Combined Annotation Dependent Depletion (CADD) tool v1.3; a scaled PHRED-like CADD score
greater than 20 was applied (11).

As the following step, based on the assumption that variants within genes intolerant to variation are
likely to be deleterious, only variants located in genes predicted to be intolerant by at least two of
the Residual Variation Intolerance Scores (RVISs) based on NHLBI-ESP6500 (12) and ExAC (9)
datasets and a local dataset, were selected. Additionally, they should be located in genes intolerant
for missense variants according to Z-score, developed by the ExAC consortium (9).

As next, the variants should locate at an evolutionary conserved position, which was evaluated by
Genomic Evolutionary Rate Profiling (GERP >2.0) (13), PhastCons (>0.3) (14) and Phylogenetic
P-value (PhyloP ≥3.0) (15) with an inclusion cutoff of at least two positive predictions.

The variants were further screened for their potential deleteriousness by using 10 different
prediction tools: Sorting Intolerant from Tolerant (SIFT) (16), Polymorphism Phenotyping version
2 (PolyPhen-2) HDIV (HumDiv) (17), PolyPhen-v2 HVAR (HumVar) (17), Log ratio test (LRT)
(18), MutationTaster (19), Mutation Assessor (20), Functional Analysis Through Hidden Markov
Models (FATHMM) (21), MetaSVM (8), MetaLR (8) and Protein Variation Effect Analyzer
(PROVEAN) (22). Variants predicted to be deleterious by at least 60% of these tools were selected
for further analyses.

**Loss-of-function variant analysis**
Frameshift, stop-gain/loss and splice-site variants affecting the canonical splice sites were considered if pedigree segregation and CADD score criteria were met. It is well known that also healthy people carry genetic variants predicted to cause loss-of function (LoF) (23). In order to discriminate pathogenic and neutral variants, we used MutPred-LOF (http://mutpredlof.cs.indiana.edu/index.html) (24). For each variant, it returns a score between zero and one; higher scores denote variants that are more likely to be pathogenic. In our analysis a threshold score of 0.50 at 5% false positive rate was used as suggested by Pagel et. al. (24). In addition, it shows up to five structural and functional mechanisms that are impacted in the affected region of the protein, accompanied by significant prior-corrected P-values. Variants that passed the filtering were further analyzed using the Translate tool (https://web.expasy.org/translate/) to translate a nucleotide (DNA/RNA) sequence to a protein sequence and IntOGen/c-BioPortal (https://www.intogen.org/search) in order to visualize the domain affected by the variant and the portion of the protein lost after the newly formed stop codon. Splice site variants were analyzed by using Human Splicing Finder (http://www.umd.be/HSF/HSF.shtml), a tool used to predict the effects of variants on splicing signals (25).

**Additional variant quality control**

Using the Integrative Genomics Viewer (IGV; version 2.4.10) (26), WGS data of all cases and controls were visually checked for correctness in order to increase the confidence of variant calls and reduce the risk of false positives.

**Germline Copy Number Variant (gCNV) analysis**

GATK gCNV module (version 4.1.7.0) was used to call CNVs from the WGS samples individually against a background of 200 WGS samples sequenced from the sample platform. The gCNVs were
called based on the best practice recommended by the GATK (https://gatk.broadinstitute.org/hc/en-us/articles/360035531152--How-to-Call-common-and-rare-germline-copy-number-variants). The major deviation was that the gCNVs were called only on the Gencode v19 exonic regions by considering them as the target regions. This decreased the turnaround time for the analysis of gCNVs from WGS data.

The resulting CNV segments with QS score above 30 were selected and annotated with the subset of gnomAD structural variant (SV) data (version 2.1, variants with ‘PASS’ filter tags and ‘DUP’ or ‘DEL’ SV types) using vcfanno (27). The segments with at least 80% overlap with a common gnomAD SV (popmax MAF > 0.1%) of same SV subtype were considered as common and removed. In addition, at least 50% of the targets (exons here) in the gCNV segments should have the denoised ploidies among the bottom (in the case on deletion) or top (in the case of duplication) 5% denoised cohort ploidies to be considered as a rare gCNVs. Subsequently, the candidate rare gCNVs were selected if they followed the disease inheritance pattern in the family.

**Protein function**

We used the UniProt Knowledgebase (UniProtKB, https://www.uniprot.org/) to evaluate the general function of the proteins, whose sequence was affected by the variants identified in our study (28).

**Details of identified candidate genes, proteins and their function**

In the main text we referred to some genes and gene variants and here we give functional details with references. The functions of the gene products were collected from the UniProtKB database and literature search.
Missense variants: genes, proteins and their function

After the FCVPPv2 application, a total of 109 potential pathogenic missense variants were identified; in most families several candidates were found and in four families none (Supplementary Table 2). All variants were private for each family, except for two genes, KIF1B (kinesin family member 1B) and DCHS1 (dachsous cadherin-related 1), in which two different missense variants were found in two unrelated families (Families 10 and 18 for KIF1B and 15 and 17 for DCHS1). KIF1B is involved in the transport of mitochondria and synaptic vesicles (29, 30). In Family 18, the variant (ENST00000263934, p.Asn1594Lys) was located between a domain of unknown function (DUF 3694) and the pleckstrin homology (PH) domain, which plays a role in recruiting proteins to different membranes and targeting them to appropriate cellular compartments. In Family 10, the variant (p.Leu181Met) was located within the kinesin motor domain. DCHS1 is a calcium-dependent cell adhesion protein. Both variants in DCHS1 (ENST00000299441, p.Arg112Gln, p.Ser667Cys) were located within one of the extracellular cadherin domains, which are thought to mediate cell-cell contacts (CADD scores were 33 and 25).

Among the other genes harboring missense variants, DAB2IP has tumor suppressor but also oncogenic properties in many solid tumors and ABL2 is an oncogene in T-cell acute lymphocytic leukemia and acute myeloid leukemia (31-33). The former has diverse signal transduction functions and it is implicated in immune processes, as are TLN1, ZFAT, CLCF1, IL11RA, SEC14L1, SAMHD1, DCST1, TPP2 and MYO1G. ZFAT and TPP2 are associated with autoimmune manifestations, and TPP2 and DCST1 with antigen presentation to T-cells; DCST1 additionally regulates type I interferon mediated innate immune response to control virus infection (34). TPP2 has been suggested to be the autoantigen target of MM and MGUS M-proteins (35).
Another group of genes with key regulatory functions constituted FOXO1, B4GALT1, and NKX3-2. FOXO1 is a member of the forkhead box family of transcription factors. It is the main target of insulin signaling and it increases osteoblast numbers and regulates B cell development (36). The protein interacts with recombination activating proteins (RAG1 and RAG2) that introduces DNA breaks at immunoglobulin genes required for V(D)J recombination in developing lymphocytes (37). FOXO1 mutations may thus contribute to aberrant RAG-dependent chromosomal translocations.

Other potentially relevant pathways include signal transduction (kinases and phosphatases), chromatin remodeling, hematopoiesis and apoptotic pathways, represented by a number of candidate genes, such as B4GALT1, NKX3-2, KMT2A and USP28. Glycosylation of immunoglobulin G (IgG) influences IgG effector functions and the addition of galactose to IgG glycans is synthesized by beta-1,4-galactosyltransferase 1, encoded by the B4GALT1 gene. Variants in this gene were associated with IgG glycosylation levels, which correlated with some autoimmune diseases and hematological neoplasms, including MM (38, 39). NKX3-2 (homeobox protein Nkx-3.2) is a member of the HOX gene transcription factors family, which are frequently dysregulated in hematologic malignancies (40). Nkx-3.2 regulates expression of chondromodulin-1 in developing cartilage and in endochondral ossification (41).

Our candidate list included two genes, KMT2A and USP28, functionally related to the recently reported MM predisposing genes, LSD1/KDM1A, encoding a lysine-specific demethylase, and USP45, an apoptosis-related gene regulating DNA repair (42, 43). KMT2A (alias MLL1) is a histone H3 lysine 4 (H3K4) methyltransferase, which plays an essential role in early development and hematopoiesis and which mediates chromatin modifications associated with epigenetic transcriptional activation (44). Somatic mutations in KMT2 gene family are reported to be among the most frequent variants in many types of cancers, including MM (45). Carcinogenic mechanism
for KMT2A mutations and the common fusion genes, which KMT2A is a part of, may be related to transcription of homeobox (HOX) target genes (45). USP28 is a deubiquitinase involved in the DNA damage-induced apoptosis. It regulates MYC protein stability in response to DNA damage (46). In MM, overexpression of MYC, mainly through complex chromosomal rearrangements, has been shown to promote myeloma cell survival and to lead to poor prognosis (47).

We checked our gene list also for the presence of the 82 somatically mutated driver genes in MM, described in Walker et al. (48) and Maura et al. (49), but only SAMHD1 passed all our in-house pipeline filters. SAMHD1 is a somatic driver in MM and the protein plays a role in maintaining dNTP levels in regulating DNA replication and damage repair and counteracting viral infections (50). It enhances immunoglobulin hypermutation in B-lymphocyte development. The present variant (ENST00000262878, Gly211Arg) maps within the histidine/aspartate domain, which possesses triphosphohydrolase activity through which SAMHD1 hydrolyzes dNTPs to deoxynucleosides (51).

**LoF variants: genes, proteins and their function**

A total of 36 LoF variants were identified in the MM families (Supplementary Table 3). If we would apply a MutPred-LOF score higher than 0.50 at a 5% false positive rate, as suggested by Pagel et al. (24), only two frameshift variants, in the genes SLC30A5 and LONP2, and six stop codon variants would pass the threshold. None of these had an apparent relationship to MM. Variants in the two genes related to immune function, IL3RA and IL17REL, had a low MutPred score. This score is not applicable to splice site variants. Of the eight splice site variants, five were predicted by Human Splicing Finder to alter the splicing motifs (indicate by ‘yes’ in Supplementary Table 3), however with no link to MM. Many of the genes with LoF mutations
encode proteins with housekeeping functions. LONP2 is an ATP-dependent protease that plays a role in maintaining peroxisome homeostasis. CSGALNACT2 is a member of the chondroitin N-acetylgalactosaminyltransferase family. HMGCLL1 is a non-mitochondrial 3-hydroxymethyl-3-methylglutaryl-CoA lyase involved in ketogenesis. FUK catalyzes the utilization of free L-fucose in glycoprotein and glycolipid synthesis.

**Copy number variants: genes, proteins and their function**

We identified seven CNVs that segregated with MM in the families (Supplementary Table 4). These CNVs affected the coding regions of 11 genes. Duplication of chr4:15936942-16178663 in Family 5 covered the genes encoding fibroblast growth factor binding proteins FGFBP1 and FGFBP2, prominin 1 (*PROM1*) involved in suppression of cell differentiation and maintenance of stem cell properties and transmembrane anterior posterior transformation protein 1 homolog (*TAPT1*). One of the primary genetic events in MM is t(4:14) translocation, creating a fusion between the immunoglobulin heavy chain (*IGH*) enhancer and *FGFR3* and leading to overexpression of *FGFR3* (52). *FGFBP1* and *FGFBP2* encode proteins that are involved in FGF ligand bioactivation by releasing them from extracellular matrix. Thus, duplication of these two genes may lead to activation of the FGF signaling, enhanced MM cell proliferation and survival and affect bone homeostasis (53, 54). *PROM1* is considered a marker of both hematopoietic progenitor and stem cells and cancer stem cells and it is overexpressed in acute lymphoblastic leukemia and many solid cancers contributing to the growth of the cancer cells (55, 56).

In a review of cancer predisposing genes it was observed that over 40% of germline variants were in genes that functioned also as somatic drivers (57). In the above, we referred to some somatic drivers, and some of the observed genes are known to interact with key signaling pathways in MM,
including PI3K/Akt/mTOR, Ras/Raf/MEK/MAPK, JAK/STAT, NF-κB, Wnt/β-catenin, and RANK/RANKL/OPG (58). Among the relevant genes in our list, DAB2IP, encoding a Ras-GTPase activating protein, modulates key oncogenic pathways such as PI3K/Akt, NF-κB, and Wnt/β-catenin (31); FOXO1 encodes for a downstream effector of Akt signaling (36); the LRP1B gene product negatively regulates the Wnt/β-catenin/TCF signaling, through its interaction with DVL2 (59).

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**Supplementary Table 1.** Summary of the variants identified in the multiple myeloma families. Number of variants after each step of Familial Cancer Variant Prioritization Pipeline version 2 is shown for each family.

| Family ID | No. cases | No possible carriers | No. healthy | MAF <0.1% | Pedigree segregation | CADD >20 | Nonsynonymous variants | Frameshift/stopgain/splice site variants | Non-coding variants | Nonsynonymous variants after pipeline | Frameshift/stopgain variants after filtering | Splice site variants after filtering |
|-----------|-----------|----------------------|-------------|-----------|----------------------|---------|-----------------------|--------------------------------------|------------------|--------------------------------------|--------------------------------------|----------------------------------|
| Family 1  | 2         | 2                    | 73103       | 8170      | 55                   | 23      | 1                     | 31                                   | 5                | 0                                    | 1                                    | 1                                |
| Family 2  | 2         | 2                    | 38348       | 12921     | 88                   | 44      | 1                     | 43                                   | 14               | 0                                    | 1                                    | 1                                |
| Family 3  | 2         | 2                    | 82691       | 1306      | 3                    | 2       | 0                     | 1                                    | 0                | 0                                    | 0                                    | 0                                |
| Family 4  | 2         | 2                    | 75478       | 3072      | 6                    | 3       | 0                     | 3                                    | 0                | 0                                    | 0                                    | 0                                |
| Family 5  | 2         | 1                    | 73995       | 1159      | 7                    | 5       | 1                     | 1                                    | 1                | 1                                    | 0                                    | 0                                |
| Family 6  | 2         | 1                    | 10699       | 33089     | 187*                 | 74      | 9                     | 101                                  | 6                | 7                                    | 2                                    | 2                                |
| Family 7  | 2         | 2                    | 72315       | 15397     | 101*                 | 53      | 6                     | 41                                   | 8                | 4                                    | 0                                    | 0                                |
| Family 8  | 4         | 2                    | 91888       | 3496      | 13                   | 5       | 1                     | 7                                    | 0                | 0                                    | 1                                    | 1                                |
| Family 9  | 3         | 2                    | 46742       | 7027      | 40#                  | 21      | 2                     | 16                                   | 6                | 0                                    | 0                                    | 0                                |
| Family 10 | 2         | 1                    | 51782       | 7223      | 48#                  | 28      | 3                     | 16                                   | 4                | 2                                    | 1                                    | 1                                |
| Family 11 | 2         | 2                    | 41753       | 12709     | 108#                 | 58      | 4                     | 45                                   | 7                | 3                                    | 1                                    | 1                                |
| Family 12 | 2         |                      | 49627       | 4815      | 28                   | 14      | 0                     | 14                                   | 3                | 0                                    | 0                                    | 0                                |
| Family 13 | 2         | 2                    | 938         | 208       | 51                   | 46      | 5                     | 0                                    | 12               | 5                                    | 0                                    | 0                                |
| Family 14 | 2         | 2                    | 862         | 20        | 4                    | 4       | 0                     | 0                                    | 0                | 0                                    | 0                                    | 0                                |
| Family 15 | 2         |                      | 626         | 197       | 46#                  | 42      | 2                     | 0                                    | 7                | 2                                    | 0                                    | 0                                |
| Family 16 | 2         | 1                    | 50077       | 6775      | 47#                  | 20      | 3                     | 23                                   | 2                | 1                                    | 0                                    | 0                                |
| Family 17 | 3         | 1                    | 73373       | 3014      | 18                   | 12      | 1                     | 5                                    | 3                | 1                                    | 0                                    | 0                                |
| Family 18 | 2         |                      | 462         | 135       | 33                   | 30      | 2                     | 1                                    | 5                | 0                                    | 0                                    | 0                                |
| Family 19 | 2         |                      | 497         | 163       | 35                   | 34      | 1                     | 0                                    | 8                | 0                                    | 1                                    | 1                                |
| Family 20 | 2         |                      | 496         | 171       | 46#                  | 37      | 6                     | 2                                    | 10               | 5                                    | 1                                    | 1                                |
| Family 21 | 2         |                      | 477         | 142       | 35                   | 33      | 1                     | 1                                    | 8                | 1                                    | 0                                    | 0                                |
MAF, minor allele frequency; CADD, Combined Annotation-Dependent Depletion
** Whole exome sequencing
* Includes 2 nonframeshift and 1 synonymous variants
# Includes 1 nonframeshift variant
µ Includes 1 synonymous variant
§ Includes 2 synonymous variant
Supplementary Table 2. Missense variants prioritized using the FCVPPv2.

| Family | GENE            | Gene name                                      | CHROM_POS_REF_ALT | Ensembl transcript:exon:nucleotide: amino acid | CADD  | ExAC z-score | Deleteriousness score (n/10)* | Function                                      |
|--------|-----------------|-----------------------------------------------|-------------------|------------------------------------------------|-------|---------------|-------------------------------|-----------------------------------------------|
| Family 1 | CNOT10 | CCR-NOT Transcription Complex Subunit 10 | 3_32776360_T_C | ENST00000328834.5:exon1:c:T1406C:p.L469S | 28.80 | 0.71          | 6                             | transcription; translation regulation           |
|         | PTPRG | Protein Tyrosine Phosphatase Receptor Type G | 3_62268487_T_C | ENST00000474889.1:exon2:c:T3998C:p.I333T | 28.10 | 0.34          | 10                            | protein phosphatase                            |
|         | BPGM | Bisphosphoglycerate Mutase                  | 7_134346548_G_A | ENST00000393132.2:exon3:c.G289A:p.G97S | 29.00 | 0.77          | 8                             | glycolysis                                     |
|         | SNX25 | Sorting Nexin 25                            | 4_186185615_T_C | ENST00000504273.1:exon4:c.T263C:p.F88S | 28.00 | 0.07          | 7                             | protein transport                              |
|         | TAB1  | TGF-Beta Activated Kinase 1 (MAP3K7) Binding Protein 1 | 22_39814820_G_A | ENST00000393132.6:exon6:c.G634A:p.E212K | 24.70 | 1.79          | 6                             | intracellular signaling                        |
| Family 2 | NADSYN1 | NAD Synthetase 1                             | 11_71201935_T_G | ENST00000319023.2:exon17:c.T1607G:p.I536S | 29.60 | 0.06          | 7                             | NAD biosynthesis/metabolism                     |
|         | TCTN1 | Tectonic Family Member 1                    | 12_111057706_G_A | ENST00000397659.4:exon2:c.G286A:p.D96N | 32.00 | 0.20          | 9                             | cilium biogenesis/degradation                  |
|         | SLC8A3 | Solute Carrier Family 8 Member A3           | 14_70633677_C_G | ENST00000381269.2:exon2:c.G1463C:p.R88S | 25.60 | 0.00          | 6                             | Ca2+ homeostasis                               |
|         | NEURL | Neuralized E3 Ubiquitin Protein Ligase 1    | 10_105344805_C_T | ENST00000369780.4:exon4:c.C1162T:p.R388C | 34.00 | 3.48          | 7                             | ubiquitination; Notch signaling                |
|         | SEMA3F | Semaphorin 3F                               | 3_50225153_C_A | ENST00000028229.3:exon19:c.C1963A:p.R655S | 34.00 | 2.68          | 7                             | neuronal development; anti-tumorigenic in endothelial cells |
|         | MYH7  | Myosin Heavy Chain 7                        | 14_23898252_A_G | ENST00000355349.3:exon1:c.T1319C:p.V440A | 25.80 | 6.54          | 9                             | muscle myosin                                 |
|         | LRP1B | LDL Receptor Related Protein 1B             | 2_141473606_G_A | ENST00000389484.3:exon37:c.C5959T:p.R1987C | 34.00 | 0.52          | 9                             | endocytosis                                    |
|         | EXOC3L4 | Exocyst Complex Component 3 Like 4         | 14_103576417_C_T | ENST00000380069.3:exon11:c.C2026T:p.R676W | 33.00 | 0.72          | 7                             | exocytosis                                     |
|         | ABCF2 | ATP Binding Cassette Subfamily F Member 2   | 7_150923514_C_T | ENST00000287844.2:exon2:c.G31A:p.A11T | 23.50 | 2.70          | 6                             | cellular transporter?                          |
|         | DAB2IP | DAB2 Interacting Protein                    | 9_124522455_G_A | ENST00000259371.2:exon6:c.G823A:p.G275R | 29.00 | 2.61          | 9                             | tumor suppressor; signal transduction; immune response |
| Gene     | Description                                      | Gene ID               | Exon | cDNA Change | Protein Change | Score | q-value | p-value | Function                                                                 |
|----------|--------------------------------------------------|-----------------------|------|-------------|----------------|-------|---------|---------|---------------------------------------------------------------------------|
| NKX3-2  | NK3 Homeobox 2                                   | 4_13543940_G_T        |      | c.C679A     | p.H227N        | 28.20 | 1.00    | 9       | skeletal development; transcription regulation                            |
| TLN1     | Talin 1                                          | 9_35725284_G_T        |      | c.C165A     | p.D55E         | 26.20 | 5.13    | 8       | cell adhesion; bone metabolism; immune response                            |
| MYH14    | Myosin Heavy Chain 14                            | 19_50752254_C_T       |      | c.C1340T    | p.A447V        | 34.00 | 1.70    | 9       | cellular myosin                                                            |
| RSPRY1   | Ring Finger And SPRY Domain Containing 1         | 16_57250905_C_T       |      | c.C65G      | p.P22R         | 23.70 | 3.71    | 7       | unknown function                                                           |
| Family 5 | BRD3                                             | 9_136918535_G_C       |      | c.C395G     | p.S132C        | 28.40 | ..      | 9       | differentiation; spermatogenesis                                           |
| Family 6 | TSNAX, TSNAX-DISC1                               | 1_231696901_C_G       |      | c.G463A     | p.G155S        | 27.90 | 0.81    | 8       | neuronal development; immune response                                     |
| UGDH     | UDP-Glucose 6-Dehydrogenase                      | 4_39523035_G_A        |      | c.G4492495  | p.G1867T       | 31.00 | 2.24    | 8       | protein modification/glycosylation                                        |
| WNT7A    | Wnt Family Member 7A                             | 3_13916462_C_T        |      | c.G1886T    | p.G629V        | 31.00 | 2.24    | 8       | neuronal development; immune response                                     |
| Family 7 | CLCF1                                            | 11_67132822_C_T       |      | c.G638G     | p.G155S        | 27.90 | 0.81    | 8       | neuronal development; immune response                                     |
| MGAT5B   | Alpha-1,6-Mannosylglycoprotein 6-Beta-N-Acetylglucosaminyltransferase B | 17_74942495_G_T       |      | c.G1886T    | p.G629V        | 31.00 | 2.24    | 8       | protein modification/glycosylation                                        |
| MYRF     | Myelin Regulatory Factor                         | 11_61539096_C_G       |      | c.C388G     | p.P280A        | 23.50 | 2.39    | 6       | oligodendrocyte differentiation                                            |
| HOXC8    | Homeobox C8                                      | 12_54404885_G_A       |      | c.G449A     | p.R150H        | 29.60 | 1.44    | 10      | cartilage differentiation                                                |
| DSP      | Desmoplakin                                      | 6_7580073_C_T         |      | c.C3650T    | p.T1217M       | 24.40 | 0.91    | 6       | desmosomal cell adhesion                                                  |
| Gene         | Protein Name                                | Chromosome | Start Position | Stop Position | Exon | Effect | p-value | q-value | Family | Function                                |
|--------------|---------------------------------------------|------------|----------------|---------------|------|--------|---------|---------|--------|------------------------------------------|
| SLC22A6      | Solute Carrier Family 22 Member 6           | 11         | 62744737_A_T   |               | ENST0000377871.3:exon9: c.T1484A:p.V495E | 24.70  | 0.04  | 7      | organic anion transport                  |
| B4GALT1      | Beta-1,4-Galactosyltransferase 1            | 9          | 33166776_G_A   |               | ENST0000379731.4:exon1: c.C392T:p.P131L | 28.70  | 1.73  | 7      | glycoconjugate and lactose biosynthesis; cell/matrix adhesion |
| SEMA5A       | Semaphorin 5A                               | 5          | 9197323_C_A    |               | ENST0000382496.5:exon1: c.G1025T:p.R342L | 34.00  | 1.68  | 6      | neurogenesis; angiogenesis               |
| Family 9     | FRY                                         | 13         | 32698466_G_A   |               | ENST0000380250.3:exon5: c.G508A:p.A170T | 33.00  | 4.03  | 7      | mitotic check-point                      |
| PDE1A        | Phosphodiesterase 1A                        | 2          | 183070764_G_C  |               | ENST0000435564.1:exon8: c.C853G:p.R285G | 34.00  | 0.45  | 10     | signal transduction                      |
| FOXO1        | Forkhead Box O1                             | 13         | 41240315_G_T   |               | ENST0000379561.5:exon1: c.C35A:p.P12Q | 26.10  | 2.77  | 9      | transcription factor; insulin signaling; bone metabolism |
| WNK3         | WNK Lysine Deficient Protein Kinase 3       | X          | 54263446_G_C   |               | ENST0000354646.2:exon2: c.C4553G:p.S1518C | 26.80  | 1.57  | 7      | electrolyte homeostasis                  |
| IL11RA       | Interleukin 11 Receptor Subunit Alpha       | 9          | 34658519_C_T   |               | ENST0000555003.1:exon8: c.C649T:p.R217C | 25.30  | 0.30  | 6      | bone metabolism; immune response         |
| SCN4A        | Sodium Voltage-Gated Channel Alpha Subunit 4 | 17         | 62018409_G_A   |               | ENST0000435607.1:exon2: c.C5233T:p.R1745C | 24.60  | 1.23  | 10     | sodium channel                           |
| Family 10    | KIF1B                                       | 1          | 10428554_C_G   |               | ENST0000263934.6:exon4: c.C6464G:p.N1548K | 31.00  | 4.04  | 6      | cellular transporter                     |
| PHACTR4      | Phosphatase And Actin Regulator 4           | 1          | 28800291_A_G   |               | ENST0000373839.3:exon7: c.A1049G:p.H350R | 22.60  | 0.56  | 6      | neurogenesis                            |
| SLC50A1      | Solute Carrier Family 50 Member 1           | 1          | 155109327_G_A  |               | ENST0000368404.4:exon3: c.G182A:p.G61E | 28.50  | 1.13  | 10     | sugar transporter                        |
| BIRC6        | Baculoviral IAP Repeat Containing 6         | 2          | 32626383_G_A   |               | ENST0000421745.2:exon7: c.G1187A:p.C396Y | 26.50  | 1.40  | 7      | apoptosis; ubiquitination                |
| Family 11    | KIAA119                                      | 15         | 81201631_G_A   |               | ENST0000394683.3:exon1: c.G1781A:p.G594D | 29.00  | 2.18  | 10     | epithelial-mesenchymal transition        |
| EPOR         | Erythropoietin Receptor                     | 19         | 11491590_A_T   |               | ENST0000222139.6:exon6: c.T797A:p.L266Q | 28.80  | 2.20  | 9      | signal transduction                      |
| ZNF236       | Zinc Finger Protein 236                     | 18         | 74589998_T_C   |               | ENST0000253159.8:exon7: c.T868C:p.C290R | 27.50  | 4.89  | 7      | transcription regulation                 |
| GRM4         | Glutamate Metabotropic Receptor 3           | 6          | 34024438_G_A   |               | ENST0000538487.2:exon6: c.C1051T:p.R351C | 34.00  | 3.48  | 10     | glutamatergic neurotransmission           |
| Gene      | Description                                      | Chromosome | Exon | Transcript ID               | Mutation         | p-value | Odds Ratio | Effect Size | Function/Effect                                      |
|-----------|--------------------------------------------------|------------|------|-----------------------------|------------------|---------|------------|-------------|-----------------------------------------------------|
| DHX16     | DEAH-Box Helicase 16                             | 6_30638221_C_T | 4    | ENST00000376442.3:exon4    | c.G632A:p.R211H  | 27.50   | 3.68       | 7           | mRNA processing; cell cycle progression              |
| FLNC      | Filamin C                                       | 7_128492756_C_T | 3    | ENST00000325888.8:exon3    | 6:c.C5954T:p.S1985L | 34.00   | 4.62       | 7           | muscle-specific actin-cross-linking protein          |
| SYNJ1     | Synaptojanin 1                                  | 21_34029139_C_T | 3    | ENST00000433931.2:exon2    | 1:c.G2770A:p.V924I | 25.90   | 1.68       | 9           | endocytosis                                          |
| TGIF2- RAB5IF Readthrough | TGFβ Induced Factor Homeobox 2, TGIF2-C20orf24 | 20_35207278_G_A | 3    | ENST00000373874.2:exon2    | c.G101A:p.R34Q  | 34.00   | ..         | 8           | transcription repression                              |
| AMPD3     | Adenosine Monophosphate Deaminase 3             | 7_10516449_C_T | 1    | ENST00000396554.3:exon8    | c.C1165T:p.R389W | 34.00   | 4.62       | 7           | muscle-specific actin-cross-linking protein          |
| BTBD2     | BTB Domain Containing 2                          | 19_1987659_C_T | 2    | ENST00000255608.4:exon6    | c.G1021A:p.E341K | 35.00   | 2.93       | 7           | protein-protein interaction                         |
| TRIM71    | Tripartite Motif Containing 71                   | 3_32932492_A_G | 5    | ENST00000383763.5:exon4    | c.A1796G:p.E599G | 24.00   | 5.59       | 10          | RNA-mediated gene silencing; ubiquitination          |
| EIF2B3    | Eukaryotic Translation Initiation Factor 2B Subunit 2 | 1_45446770_G_A | 2    | ENST00000360403.2:exon2    | c.C71T:p.P24L   | 32.00   | 0.56       | 10          | protein biosynthesis                                 |
| BBS2      | Bardet-Biedl Syndrome 2                          | 16_56540081_T_C | 1    | ENST00000245157.5:exon6    | c.A668G:p.N223S  | 23.70   | 0.72       | 9           | cilium biogenesis/degradation; protein transport     |
| KMT2A     | Lysine Methyltransferase 2A                      | 11_118376242_C_T | 3    | ENST00000534358.1:exon2    | 7:c.C9635T:p.T3212I| 23.20   | 6.64       | 6           | histone modification; hematopoiesis                  |
| DLGAP4    | DLG Associated Protein 4                         | 20_35060539_G_A | 3    | ENST00000373913.3:exon3    | c.G419A:p.R140H | 31.00   | 3.26       | 7           | molecular organization of synapses; neuronal cell signaling |
| INTS5     | Integrator Complex Subunit 5                    | 11_62415289_C_T | 2    | ENST00000330574.2:exon2    | c.G2263A:p.G755S | 29.60   | 2.28       | 7           | snRNA transcription and processing                   |
| HSPB1     | Heat Shock Protein Family B (Small) Member 1     | 7_75932109_G_C | 2    | ENST00000248553.6:exon1    | c.G80C:p.R27P   | 31.00   | 1.54       | 9           | molecular chaperone                                  |
| TLE1      | TLE Family Member 1, Transcriptional Corepressor | 9_84228369_G_A | 1    | ENST00000376499.3:exon1    | 2:c.C986T:p.P329L| 25.70   | 3.23       | 7           | transcriptional corepressor                          |
| SEC14L1   | SEC14 Like Lipid Binding 1                      | 17_75205479_A_G | 2    | ENST00000436233.4:exon1    | 4:c.A1532G:p.E511G| 34.00   | 2.60       | 7           | signal transduction inhibition; innate immunity      |
| THR8      | Thyroid Hormone Receptor 8                      | 3_24185122_T_C | 2    | ENST00000396671.2:exon8    | c.A608G:p.E203G | 24.40   | 2.84       | 10          | transcription regulation                             |
| Gene Symbol | Gene Name | Chromosome | Transcript ID | Mutation Details | Log R ratio | | | Function |
| --- | --- | --- | --- | --- | --- | | | |
| UFC1 | Ubiquitin-Fold Modifier Conjugating Enzyme 1 | 1_161123804_C_T | ENST00000368003.5:exon1: c.C17T:p.T6M | 32.00 | 0.54 | 7 | ubiquitination |
| STARD10 | STAR Related Lipid Transfer Domain Containing 10 | 11_72466798_C_T | ENST00000334805.6:exon6: c.G578A:p.G193D | 32.00 | 1.78 | 6 | lipid transporter |
| COL5A2 | Collagen Type V Alpha 2 Chain | 2_189957140_G_A | ENST00000334805.6:exon6: c.G578A:p.G193D | 32.00 | 1.78 | 6 | lipid transporter |
| SAMHD1 | SAM And HD Domain Containing Deoxynucleoside Triphosphohydrolase 1 | 20_35555650_C_T | ENST00000326878.4:exon6: c.G631A:p.G211R | 34.00 | 2.20 | 10 | immune response |
| DCHS1 | Dachsous Cadherin-Related 1 | 11_6662510_C_T | ENST00000299441.3:exon2: c.G335A:p.R112Q | 33.00 | 2.34 | 6 | calcium-dependent cell adhesion |
| KLHDC3 | Kelch Domain Containing 3 | 6_42986424_C_T | ENST00000326974.4:exon7: c.C787T:p.R263W | 35.00 | 3.83 | 7 | meiotic recombination |
| ATLAS | Atlastin GTPase 3 | 11_63419412_A_C | ENST00000398868.3:exon5: c.T557G:p.L186R | 29.20 | 0.82 | 10 | endoplasmic reticulum tubular network biogenesis |
| WFS1 | Wolframin ER Transmembrane Glycoprotein | 4_6296854_G_A | ENST00000226760.1:exon7: c.G799A:p.D267N | 24.10 | | 6 | Ca2+ homeostasis |
| FGG | Fibrinogen Gamma Chain | 4_15553353_C_T | ENST00000336098.3:exon3: c.G124A:p.G42S | 32.00 | 0.28 | 10 | hemostasis |
| DCST1 | DC-STAMP Domain Containing 1 | 1_155006565_C_T | ENST00000295542.1:exon2: c.C537T:p.R18L | 25.90 | 0.41 | 6 | immune response |
| SLC12A7 | Solute Carrier Family 12 Member 7 | 5_1087125_A_G | ENST00000264930.5:exon6: c.T568C:p.S190P | 24.80 | 1.65 | 10 | ion transport |
| KIF1B | Kinesin Family Member 1B | 1_10327549_C_A | ENST00000317370.8:exon8: c.G973A:p.A325T | 27.10 | 4.04 | 8 | cellular transporter |
| SLC2A4 | Solute Carrier Family 2 Member 4 | 17_7188211_G_A | ENST00000326934.6:exon6: c.C541A:p.L181M | 29.50 | 0.64 | 9 | glucose transporter |
| Gene            | Description                                                   | Accession Numbers                                                                 | Exon | Chromosome | Position (bp) | GO Terms                                      |
|-----------------|---------------------------------------------------------------|----------------------------------------------------------------------------------|------|------------|--------------|-----------------------------------------------|
| ATP11A          | ATPase Phospholipid Transporting 11A                          | ENST00000487903.1:exon2:ENST00000487903.1:exon2                                 | 12   | 13         | 113512584    | 24.50 1.44 10 lipid transporter               |
| ITPRP           | Inositol 1,4,5-Trisphosphate Receptor Interacting Protein     | ENST00000278071.2:exon3:ENST00000278071.2:exon3                                 | 6    | 10         | 106074540    | 31.00 0.39 6 intracellular calcium signaling   |
| GPR125          | ADGRA3; Adhesion G Protein-Coupled Receptor A3                 | ENST00000334004.5:exon1:ENST00000334004.5:exon1                                 | 5    | 4          | 22390532     | 26.20 0.73 7 signal transduction              |
| Family 19       | ABL2 ABL Proto-Oncogene 2, Non-Receptor Tyrosine Kinase        | ENST00000502732.1:exon4:ENST00000502732.1:exon4                                 | 9    | 11         | 179095661    | 34.00 1.31 10 cell growth and survival        |
| PDCD11          | Programmed Cell Death 11                                       | ENST00000369797.3:exon1:ENST00000369797.3:exon1                                 | 2    | 10         | 105173778    | 26.00 0.56 7 rRNA processing                 |
| Family 20       | CHD3 Chromodomain Helicase DNA Binding Protein 3               | ENST00000330494.7:exon3:ENST00000330494.7:exon3                                 | 6    | 17         | 78102277     | 26.10 7.15 10 chromatin remodeling            |
| ATP2B2          | ATPase Plasma Membrane Ca2+ Transporting 2                    | ENST00000360273.2:exon2:ENST00000360273.2:exon2                                 | 10   | 3          | 10381937     | 22.90 6.36 7 Ca2+ homeostasis                |
| OIT3            | Oncoprotein Induced Transcript 3                              | ENST00000334011.5:exon7:ENST00000334011.5:exon7                                 | 10   | 10         | 74684222     | 24.10 0.73 10 liver development and function  |
| DAAM1           | Dishevelled Associated Activator Of Morphogenesis 1           | ENST00000395125.1:exon2:ENST00000395125.1:exon2                                 | 5    | 14         | 59835503     | 34.00 1.22 10 cell polarity; Wnt signaling    |
| SLC6A19         | Solute Carrier Family 6 Member 19                             | ENST00000304460.10:exon3:ENST00000304460.10:exon3                               | 10   | 5          | 1210676      | 27.60 0.37 8 amino acid transport             |
| Gene       | Description                        | Chromosome | Position | Reference Allele | Alternative Allele | CADD Score | PolyPhen-2 HDIV Score | ExAC MAF | Function                  |
|------------|------------------------------------|------------|----------|------------------|--------------------|------------|-----------------------|----------|---------------------------|
| MYO1G      | Myosin IG                          | 7_45006305 | C_T      | ENST00000258787.7:exon1:5:c.G1915A:p.A639T | ENST00000258787.7:exon1:5:c.G1915A:p.A639T | 33.00      | 1.83                  | 9        | immune response           |
| DNAH2      | Dynein Axonemal Heavy Chain 2      | 17_7691226 | A_T      | ENST00000572933.1:exon1:3:c.A6652T:p.T2218S | ENST00000572933.1:exon1:3:c.A6652T:p.T2218S | 24.20      | 2.44                  | 10       | motor protein             |
| DARS2      | Aspartyl-tRNA Synthetase, Mitochondrial | 1_173800731 | G_T      | ENST00000361951.4:exon5: c.G455T:p.C152F | ENST00000361951.4:exon5: c.G455T:p.C152F | 27.20      | 0.56                  | 8        | tRNA aminoacylation; protein biosynthesis |
| ATP1B2     | ATPase Na+/K+ Transporting Subunit Beta 2 | 17_7559161 | G_A      | ENST00000250111.4:exon7: c.G821A:p.R274Q | ENST00000250111.4:exon7: c.G821A:p.R274Q | 29.00      | 0.64                  | 6        | ion transport             |
| RAPGEF4    | Rap Guanine Nucleotide Exchange Factor 4 | 2_173832028 | A_G      | ENST00000397081.3:exon1:0:c.A860G:p.Y287C | ENST00000397081.3:exon1:0:c.A860G:p.Y287C | 27.90      | 1.55                  | 9        | exocytosis                |
| ADAT3      | Adenosine Deaminase TRNA Specific 3 | 19_1912227 | G_T      | ENST00000329478.2:exon2: c.G181T:p.A61S | ENST00000329478.2:exon2: c.G181T:p.A61S | 24.70      | 1.42                  | 7        | tRNA processing           |
| MORN4      | MORN Repeat Containing 4           | 10_99379394 | C_A      | ENST00000307450.6:exon2: c.G17T:p.G6V | ENST00000307450.6:exon2: c.G17T:p.G6V | 34.00      | 0.22                  | 8        | response to axon injury   |
| DDX59      | DEAD-Box Helicase 59               | 1_200619764 | A_G      | ENST00000331314.6:exon5: c.T1103C:p.L368P | ENST00000331314.6:exon5: c.T1103C:p.L368P | 29.50      | 0.08                  | 6        | RNA metabolism            |
| USP28      | Ubiquitin Specific Peptidase 28    | 11_113683078 | C_A      | ENST0000003302.4:exon1:6:c.G1892T:p.R631I | ENST0000003302.4:exon1:6:c.G1892T:p.R631I | 34.00      | 0.17                  | 7        | DNA damage-induced apoptosis |
| GALNT10    | Polypeptide N-Acetylgalactosaminyltransferase 10 | 5_153783769 | C_T      | ENST00000297107.6:exon8: c.C1162T:p.R388W | ENST00000297107.6:exon8: c.C1162T:p.R388W | 35.00      | 1.75                  | 9        | synthesis of mucin-type oligosaccharides |
| ABTB2      | Ankyrin Repeat And BTB Domain Containing 2 | 11_34378544 | C_A      | ENST00000435224.2:exon1: c.G587T:p.G196V | ENST00000435224.2:exon1: c.G587T:p.G196V | 28.40      | 1.16                  | 7        | hepatocyte growth         |
| PCDHGC5    | Protocadherin Gamma Subfamily C, 5 | 5_140870047 | C_T      | ENST00000252087.1:exon1: c.C1240T:p.R414W | ENST00000252087.1:exon1: c.C1240T:p.R414W | 31.00      | 1.59                  | 7        | call adhesion in brain    |
| KLHL18     | Kelch Like Family Member 18        | 3_47376277 | T_A      | ENST00000232766.5:exon6: c.T866A:p.L289H | ENST00000232766.5:exon6: c.T866A:p.L289H | 25.30      | 2.74                  | 7        | ubiquitination; mitotic check-point |

CHROM_POS_REF_ALT, chromosome_position_reference allele_alternative allele; CADD, Combined Annotation-Dependent Depletion; ExAC, the Exome Aggregation Consortium

*Deleteriousness of the variants were predicted using Sorting Intolerant from Tolerant (SIFT), Polymorphism Phenotyping version-2 (PolyPhen-2) HDIV (HumDiv), PolyPhen-v2 HVAR (HumVar), Log ratio test (LRT), MutationTaster, Mutation Assessor, Functional Analysis Through Hidden Markov Models (FATHMM), MetaSVM, MetaLR, Protein Variation Effect Analyzer (PROVEAN)
**Supplementary Table 3** Loss-of-function variants segregating with the disease in the multiple myeloma families.

| Family ID | GENE | Gene name | CHROM_POS_REF_ALT | VARIANT CLASSIFICATION | Ensembl transcript;exon:nucleotide: amino acid or Ensembl transcript;HGVS* | CADD | Impact on protein (MutPred-LOF; Human Splicing finder)** | Function |
|-----------|------|-----------|-------------------|------------------------|--------------------------------------------------------------------------------|------|--------------------------------------------------------|----------|
| Family 1  | NPFFR2 | Neuropeptide FF Receptor 2 | 4_73003756_G_A | splicing | ENST00000308744.6; HGVSp:c.635-1G>A | 26.40 | yes | G-protein-coupled receptor signaling; pain modulation |
| Family 2  | KRI1 | KRI1 Homolog | 19_10673404_C_A | splicing | ENST00000312962.6; HGVSp:c.401+1G>T | 25.40 | yes |  |
| Family 6  | FUK | Fucose Kinase | 16_70500783_A_G | splicing | ENST00000288078.6; HGVSp:c.412-2A>G | 23.40 | yes | glycoprotein and glycolipid synthesis |
|           | U2AF1L4 | U2 Small Nuclear RNA Auxiliary Factor 1 Like 4 | 19_36234717_TG_T | frameshift deletion | ENST00000378975.3:exon5:c.453delC:p.P151fs | 24.20 | 0.29 | mRNA processing/splicing |
|           | AHI1 | Abelson Helper Integration Site 1 | 6_135752371_G_C | stopgain SNV | ENST00000367800.4:exon15:c.C2348G:p.S783X | 38.00 | 0.53 | cilium biogenesis/degradation |
|           | DNLZ | DNL-Type Zinc Finger | 9_139258036_C_T | stopgain SNV | ENST00000371738.3:exon1:c.G131A:p.W44X | 35.00 | 0.33 | chaperone |
|           | MYO19 | Myosin XIX | 17_34867295_C_G | splicing | ENST00000614623.4; HGVSp:c.897-1C>G | 25.00 | yes | motor protein |
|           | MUC17 | Mucin 17, Cell Surface Associated | 7_100679568_CA_C | frameshift deletion | ENST00000306151.4:exon3:c.4872delA:p.S1624fs | 23.30 | 0.4 | homeostasis of mucosal surfaces |
|           | IARS1 | Isoleucyl-tRNA Synthetase 1 | 9_95004514_T_TATGA | frameshift insertion | ENST00000375643.3:exon29:c.3097_3098insTCAT:p.I1033fs | 35.00 | 0.42 | aminoacyl-tRNA synthetase |
|           | CORIN | Corin, Serine Peptidase | 4_47667210_A_C | stopgain SNV | ENST00000273857.4:exon11:c.T1428G:p.Y476X | 36.00 | 0.62 | serine protease |
|           | LONP2 | Lon Peptidase 2, Peroxisomal | 16_48311302_TG_T | frameshift deletion | ENST00000285737.4:exon8:c.1296delG:p.V432fs | 22.60 | 0.68 | peroxisome homeostasis |
| Family 7  | IFT74 | Intraflagellar Transport 74 | 9_26990150_C_T | stopgain SNV | ENST00000443698.1:exon8:c.C544T:p.R182X | 41.00 | 0.43 | cilium biogenesis/degradation |
|           | ELOVL2 | ELOVL Fatty Acid Elongase 2 | 6_10990562_G_A | stopgain SNV | ENST00000354666.3:exon6:c.C619T:p.Q207X | 40.00 | 0.56 | fatty acid biosynthesis |
| Family | Gene             | Description                           | ENST_ID | Exon Region | SNP Type | Effect | Freq | Function                                      |
|--------|------------------|---------------------------------------|---------|-------------|----------|--------|------|-----------------------------------------------|
| Family 1 | RESP18           | Regulated Endocrine Specific Protein 18 | ENST0000333527.5 | exon2:c.184delC:p.L62fs | frameshift deletion | 26.00  | 0.27 | regulatory role in corticotrophs               |
|         | CDH19            | Cadherin 19                           | ENST0000331035.4 | exon6:c.591dupT:p.D197fs | frameshift deletion | 25.70  | 0.47 | cell adhesion                                  |
| Family 8 | CENPO            | Centromere Protein O                  | ENST00003333527.5 | exon2:c.184delC:p.L62fs | frameshift deletion | 25.30  | no   | mitotic check-point                           |
| Family 10 | IL3RA            | Interleukin 3 Receptor Subunit Alpha   | ENST0000331035.4 | exon6:c.591dupT:p.D197fs | frameshift deletion | 22.70  | 0.44 | immune response                               |
|         | IL17REL          | Interleukin 17 Receptor E Like         | ENST00003333527.5 | exon2:c.184delC:p.L62fs | frameshift deletion | 27.90  | 0.37 | interleukin 17 receptor activity              |
| Family 11 | CRYL1            | Crystallin Lambda 1                   | ENST00003333527.5 | exon2:c.184delC:p.L62fs | frameshift deletion | 37.00  | glucose catabolism                            |
|         | ARHGAP40         | Rho GTPase Activating Protein 40      | ENST00003333527.5 | exon2:c.184delC:p.L62fs | frameshift deletion | 24.20  | no   | GTPase activation                             |
|         | ABCC11           | ATP Binding Cassette Subfamily C Member 11 | ENST00003333527.5 | exon2:c.184delC:p.L62fs | frameshift deletion | 37.00  | 0.39 | transport of small molecules/multidrug resistance |
| Family 13 | GHSR             | Growth Hormone Secretagogue Receptor  | ENST00003333527.5 | exon2:c.184delC:p.L62fs | frameshift deletion | 36.00  | 0.45 | G-protein coupled receptor; growth hormone secretion|
|         | SLC30A5          | Solute Carrier Family 30 Member 5     | ENST00003333527.5 | exon2:c.184delC:p.L62fs | frameshift deletion | 35.00  | 0.55 | zink transport                                |
|         | ARHGEF19         | Rho Guanine Nucleotide Exchange Factor 19 | ENST00003333527.5 | exon2:c.184delC:p.L62fs | frameshift deletion | 37.00  | 0.44 | GTPase activation                             |
|         | CLSPN            | Claspin                               | ENST00003333527.5 | exon2:c.184delC:p.L62fs | frameshift deletion | 34.00  | 0.44 | cell cycle regulation                         |
| Family 15 | NBAS             | NBAS Subunit Of NRZ Tethering Complex | ENST00003333527.5 | exon2:c.184delC:p.L62fs | frameshift deletion | 43.00  | 0.49 | Golgi to endoplasmic reticulum transport      |
|         | CSGALNACT2       | Chondroitin Sulfate N-Acetylgalactosaminyltransferase 2 | ENST00003333527.5 | exon2:c.184delC:p.L62fs | frameshift deletion | 47.00  | 0.54 | chondroitin sulfate synthesis                 |
| Family 16 | CNGA1            | Cyclic Nucleotide Gated Channel Subunit Alpha 1 | ENST00003333527.5 | exon2:c.184delC:p.L62fs | frameshift deletion | 25.90  | 0.49 | phototransduction                             |
|         | DPYD             | Dihydropyrimidine Dehydrogenase       | ENST00003333527.5 | exon2:c.184delC:p.L62fs | frameshift deletion | 36.00  | 0.39 | pyrimidine catabolism                         |
| Family | Gene        | Description                          | CHROM_POS_REF_ALT | SPlice Site | CADD  | LOF     | Phenotype                  |
|--------|-------------|--------------------------------------|-------------------|-------------|-------|---------|---------------------------|
| 19     | TMPRSS15    | Transmembrane Serine Protease 15     | 21_19715821_A_C   | splicing    |       | 24.00   | activation of pancreatic proteolytic proenzymes |
| 20     | COL19A1     | Collagen Type XIX Alpha 1 Chain      | 6_70854808_A_G    | splicing    |       | 23.20   | cell adhesion              |
|        | POP5        | POP5 Homolog, Ribonuclease P/MRP Subunit | 12_121018949_GA_G | frameshift deletion | ENST00000357500.4:exon2:c.131delT:p.F44fs | 33.00 | 0.35 | rRNA/tRNA processing |
|        | HMGCLL1     | 3-Hydroxymethyl-3-Methylglutaryl-CoA Lyase Like 1 | 6_55406595_G_A | stopgain SNV | ENST00000398661.2:exon4:c.C319T:p.R107X | 36.00 | 0.57 | ketogenesis |
|        | CARF        | Calcium Responsive Transcription Factor | 2_203842036_GA_G | frameshift deletion | ENST00000402905.3:exon13:c.1540delA:p.T514fs | 33.00 | 0.35 | transcription regulation |
|        | CATSPER3    | Cation Channel Sperm Associated 3    | 5_134345082_C_T   | stopgain SNV | ENST00000282611.6:exon6:c.C838T:p.R280X | 35.00 | 0.52 | voltage-gated calcium channel |
|        | SHPK        | Sedoheptulokinase                    | 17_3527481_G_A    | stopgain SNV | ENST00000225519.3:exon3:c.C355T:p.R119X | 23.80 |       | glucose metabolism        |

CHROM_POS_REF_ALT, chromosome_position_reference allele_alternative allele; CADD, Combined Annotation-Dependent Depletion; LOF, loss-of-function;
* For LOF variants (frameshift and stopgain), Ensembl transcript:exon:nucleotide change:amino acid change are shown. For splice site variants Ensemble transcript; Human Genome Variation Society (HGVS) sequence variant nomenclature is used.
** For LOF variants (frameshift and stopgain), pathogenic and neutral variants were predicted using MutPred-LOF (http://mutpredlof.cs.indiana.edu/index.html) with a threshold score of 0.50 at 5% false positive rate. Human Splicing Finder (http://www.umd.be/HSF/HSF.shtml) was used to evaluate the effect of splice site variants, with yes/no score.
**Supplementary Table 4.** Copy number variants segregating with the disease in multiple myeloma families.

| Family ID | CHROM_START_END          | Type (size) | GENE | Gene name                          | Function                                                                 |
|-----------|--------------------------|-------------|------|------------------------------------|--------------------------------------------------------------------------|
| Family 5  | 4_15936942_16178663      | DEL (241.72 kb) | FGFBP1 | Fibroblast Growth Factor Binding Protein 1 | cell proliferation, differentiation and migration                         |
|           |                          |             |      | FGFBP2                             | Fibroblast Growth Factor Binding Protein 2                                |
|           |                          |             |      | PROM1                              | Prominin 1                                                                |
|           |                          |             |      | TAPT1                              | Transmembrane Anterior Posterior Transformation 1                        |
| Family 7  | 22_30889887_30892208     | DUP (2.32 kb) | SEC14L4 | SEC14 Like Lipid Binding 4          | transport of hydrophobic ligands                                          |
| Family 7  | 3_43641626_43647605      | DEL (5.98 kb) | ANO10 | Anoctamin 10                       | calcium-activated chloride channel                                        |
|           | 12_50363908_50364605     | DEL (0.68kb) | AQP6 | Aquaporin 6                        | kidney-specific water channel                                              |
| Family 10 | 1_151028877_151033279    | DUP (4.40 kb) | MLLT11 | MLLT11 Transcription Factor 7 Cofactor | regulation of lymphoid development                                        |
|           |                          |             |      | CDC42SE1                           | CDC42 Small Effector 1                                                    |
| Family 11 | 10_126172458_151033279   | DEL (0.69kb) | LHPP | Phospholysine Phosphohistidine Inorganic Pyrophosphate Phosphatase | phosphatase                                                               |
| Family 17 | 1_87029094_87039091      | DEL (10.00 kb) | CLCA4 | Chloride Channel Accessory 4       | calcium-activated chloride conductance                                    |

DEL, deletion; DUP, duplication
Supplementary Figure 1. Pedigrees of the multiple myeloma families. For cancer patients age of diagnosis is shown, for healthy family members age at sampling. Families 18-21 consisted of two first- or second-degree relatives diagnosed with MM and are not shown.
Family 8

Family 9

Family 10

Family 11

Family 12

Family 13

Family 14

Family 15

Family 16

Family 17

- Multiple myeloma
- MGUS
- Multiple myeloma or MGUS and AL amyloidosis
- Plasmacytoma or aberrant plasma cell clone in bone marrow
- Other cancer
- * WGS performed
- † Age at death