Tumor 1			-									
Chromosome	Start position	End position	Reference	Variant	Reads	Variatior reads	Gene name	Reference Amino Acid	Mutation Amino Acid	Synonymous	Amino Acid position	mRNA changes
chr7	101265374	101265374	A	Т	95	2	21 MYL10			FALSE	-1	
chr19	54444763	54444763	G	А	49		1 CACNG7	S	Ν	FALSE	155	464G>A
Tumor 2												
								Reference	Mutation		Amino	
						Variation	Gene	Amino	Amino		Acid	mRNA
Chromosome	Start position	End position	Reference	Variant	Reads	Variatior reads	Gene name	Amino Acid	Amino Acid	Synonymous	Acid position	mRNA changes
Chromosome chr1	Start position 93202030	End position 93202030	Reference G	Variant	Reads 85	Variation reads	Gene name 8 EVI5	Amino Acid A	Amino Acid D	Synonymous FALSE	Acid position 69	mRNA changes 206C>A
Chromosome chr1 chr5	Start position 93202030 132652300	End position 93202030 132652300	Reference G G	Variant T A	Reads 85 93	Variation reads	Gene name EVI5 5 FSTL4	Amino Acid A L	Amino Acid D F	Synonymous FALSE FALSE	Acid position 69 152	mRNA changes 206C>A 454C>T
Chromosome chr1 chr5 chr7	Start position 93202030 132652300 128517835	End position 93202030 132652300 128517835	Reference G G C	Variant T A T	Reads 85 93 32	Variation reads	Gene name EVI5 5 FSTL4	Amino Acid A L	Amino Acid D F	Synonymous FALSE FALSE FALSE	Acid position 69 152 -1	mRNA changes 206C>A 454C>T
Chromosome chr1 chr5 chr7 chr11	Start position 93202030 132652300 128517835 69063542	End position 93202030 132652300 128517835 69063542	Reference G G C G	Variant T A T A	Reads 85 93 32 123	Variation reads	 Gene name EVI5 FSTL4 KCP MYEOV 	Amino Acid A L G	Amino Acid D F R	Synonymous FALSE FALSE FALSE FALSE	Acid position 69 152 -1 209	mRNA changes 206C>A 454C>T 625G>A
Chromosome chr1 chr5 chr7 chr11 chr14	Start position 93202030 132652300 128517835 69063542 20345206	End position 93202030 132652300 128517835 69063542 20345206	Reference G G C G G G	Variant T A T A T T	Reads 85 93 32 123 301	Variation reads	Gene name 28 EVI5 35 FSTL4 16 KCP 15 MYEOV 31 OR4K2	Amino Acid A L G W	Amino Acid D F R C	Synonymous FALSE FALSE FALSE FALSE FALSE	Acid position 69 152 -1 209 260	mRNA changes 206C>A 454C>T 625G>A 780G>T
Chromosome chr1 chr5 chr7 chr11 chr14 chr19	Start position 93202030 132652300 128517835 69063542 20345206 52272633	End position 93202030 132652300 128517835 69063542 20345206 52272633	Reference G C C G G G G G	Variant T A T A T A A	Reads 85 93 32 123 301 112	Variation reads	Gene name 28 EVI5 35 FSTL4 16 KCP 45 MYEOV 31 OR4K2 40 FPR2	Amino Acid A L G W R	Amino Acid D F R C Q	Synonymous FALSE FALSE FALSE FALSE FALSE FALSE	Acid position 69 152 -1 209 260 241	mRNA changes 206C>A 454C>T 625G>A 780G>T 722G>A
Chromosome chr1 chr5 chr7 chr11 chr14 chr14 chr19 chr22	Start position 93202030 132652300 128517835 69063542 20345206 52272633 42053003	End position 93202030 132652300 128517835 69063542 20345206 52272633 42053003	Reference G G C G G G A	Variant T A T A T A G	Reads 85 93 32 123 301 112 37	Variation reads	Gene name 28 EVI5 35 FSTL4 16 KCP 15 MYEOV 31 OR4K2 40 FPR2 18 XRCC6	Amino Acid A L G W R K	Amino Acid D F R C Q R	Synonymous FALSE FALSE FALSE FALSE FALSE FALSE FALSE	Acid position 69 152 -1 209 260 241 463	mRNA changes 206C>A 454C>T 625G>A 780G>T 722G>A 1388A>G

Table S1. Exome sequencing reveals variants in tumors from P3. Tumor 1 represents the exome sequencing results from the osteosarcoma, Tumor 2 represents results from the AML. Not shown is the *RPL9* variant, which was found in both tumor types.



Figure S1 (related to Figure 1 and Table 1). Colon tissue biospy revealing TP53 stabilization in a DBA-affected individual with colitis carrying the *RPL9* c.-2+1G>C variant. A) Haematoxylin and eosin staining of biopsied colon tissue taken from a DBA-affected individual or a healthy age-matched control. Arrows point to nuclear fragmentation typical of apoptotic bodies.
B) Immunohistochemistry of biopsied colon tissue stained with antibody against TP53 (healthy control left panel, DBA-affected individual with the *RPL9* variant center and left panels). Note intense TP53 staining in the epithelial cells in basal half of the crypts. C) Imunohistochemistry of biopsied colon tissue stained with antibodies against CD3 (healthy control left panel, DBA-affected individual with the *RPL9* variant center and left panel, DBA-affected individual with the *RPL9* variant center and left panel, DBA-affected individual with the *RPL9* variant center and left panel, DBA-affected individual with the *RPL9* variant center and left panel, DBA-affected individual with the *RPL9* variant center and left panel). Arrowheads indicate CD3+ cells infiltrating the epithelium.



Figure S2. *RPL9* **varaints are found in individuals P2 and P3.** Integrated genomics viewer (IGV) screen shots show the exome sequencing results of the different tissues from P2 and P3. From top to bottom P2 germ cell, P3 osteosarcoma, and P3 AML.

Leu20	
1	80
S. cerevisiae MKYIQTEQQIEVPEGVTVSIKSRIVKVVGPRGTLTKNLKHIDVTFTKVNN-QLIKVAVHNGGRKHVAALRTVKSLVDN	Ŭ
C. elegans <mark>maliesndtvvfpegvtftvanr</mark> ivevtgprgtirkdfrelememerigk <mark>-stlrvrkwfgvrkelaairtvc</mark> seiknm	i
nelanogaster <mark>mrtinsno<mark>cvk</mark>ipkdikasvkarvvtitgtrgtikrsfkhlaldmymp <mark>– dkrtikvekwfgtkkelaavrtvc</mark>shienm</mark>	1
D. reriowktilsnotvdipdnvtvslkgrtvtvkgprgvlrrefnhinlelsligkkokklrvdkwwgnrkelatvrtigsevon	4
X. aevis matilsnqivdipqnvdislagatvivagprgvlranfneinvelclmgakkarlrvdkwwgnrkelatvrticsevqnm	4
G. gallus MKTILSNQTVDIPEQVSVSLKGRTVIVKGPRGTLRRDFNHINVELSLLGKKRRKLRVDKWWGNRKELATVRTICSEVQNM	4
H. sapiens METIL SNOT VDIPEN VDITIKCETVIV KOPROTIERDENE IN VELSILG KKKKEIR VDKWMCNEKELATVETICSEVON	ł
M. musculus METILSNQAVDIPENVETALEGRAVIVEGPRGALERDENEINVELSLLGEKKKER RVDKWGNERELAAVRAICSEVQN	4
01	160
	100
	t i
nelanogaster i kov te cevek ravya he pin ov te sennt vielen piceky i reveka povovnista okobel i ve ond tesvece	1
D. rerio IKGVILGFRYKMRSVYAHFPINVVI-OESGSLVEIRNFLGEKYIRRVRMROGVACAVSAAOKDELVLEGNDIELVSNSA	[
X. Jaevis v Kovilo Frykmrsvya HFPINVVI OESOSLVEI RNFLOEKYI RRVRMRSOVACTVSOA OKDELILEGNDI ELVSNSA	
G. gallus ikgvilg frykmrsvya hfpinvvi odnoslve irnfloeky irrvrmrpgvs cavsoa okdelilegnd i elvsnsa	1
H. sapiens irgvilgfryrmrsvyaefpinvvi - gengslveirnflgeryirrvrmrpgvacsvsgagrdelilegndielvsnsa	1
M. MUSCULUS IKGVILGFRYKMRSVYAHFPINVVI QENGSLVEIRNFLGEKYIRRVRMRIGVACSVSQAQKDELILEGNDIELVSNSA	
161 181	
S. cerevisiae ADLQQICRVRNKDIRKFLDGIYVSHKCFITEDL	
C. elegans ARIQQSTAVKEKDIRKFLDGIYVSEKTTIVPTD	
D revie	
M. musculus aligoatty knkdirkfldgiyysek tvoqade-	

Figure S3 (related to Figure 1). Multiple sequence alignment (MSA) of uL6 proteins.

The human Leu20 residue in uL6 (*RPL9*) precedes a universally conserved Lys21 residue (indicated with *) and is itself conserved in mammals, birds, frogs, and fish. The hydrophobicity of residue 20 is also conserved in eukaryotes, as yeast, worms, and flies reveal either Ile20 or Val20 in this position. Color coding is based on the physicochemical properties of the amino acids, with hydrophobic amino acids shown in green (black font), large hydrophobic in green (white font), negatively charged in dark blue, positively charged in red, cysteines in yellow, polar in purple, and small alcohol in light blue.



Figure S4 (related to Figure 2). A schematic of pre-rRNA processing in human cells. Major endonucleolytic cleavage steps are labeled with black circles, the relevent enzymes are also noted.



Figure S5 (related to Figures 2 and 3). Reductions of *RPL9* levels by siRNAs in HeLa cells reveal pre-rRNA processing defects and impair 60S ribosomal subunit formation.
A) Northern blot analysis of HeLa cells transfected with scrambled control siRNAs or siRNAs against *RPL9* mRNA. Probes used are against ITS2, ITS1-5.8S, and 18S-ITS1 (5'ITS1).
B) Quantitative RAMP analysis of (A) (three independent experiments). C) Polysome profile analysis of HeLa cells transfected with scrambled control siRNAs against *RPL9*. The small (40S) and large (60S) ribosomal subunits are labeled as well as the 80S monosome and the polysomes. Note the strong reduction of the 60S peaks in the siRPL9 samples.



Figure S6 (Related to Figure 5). FACS plots and western blots of cells in red cell culture assays.
A-B) Day 7 (A) and Day 13 (B) Annexin V/DAPI staining of healthy control and *RPL*9 c.-2+1 cells.
C) Quantification of (A) and (B). D) Day 7 CD34+/CD36+ staining of healthy control and *RPL*9 c.-2+1 cells.
E) Quantification of (D). F) Western blot of cells at Day 7 with antibodies against TP53 and CDKN1A (p21).



Figure S7. FACS analysis of cells derived P2 carrying the uL6 p.Leu20Pro variant compared to a healthy control in red cell culture assays. A) Cells stained with antibodies against Band3 at Days 10 and 12. **B)** Cells stained with antibodies against GPA at Days 10 and 12.



Figure S8 (related to Figure 7). Cell-based assays measuring translational fidelity. A) +1 Programmed Ribosomal Frameshifting (PRF) levels in LCLs derived from individuals carrying variants in *RPL9* compared to LCLs derived from two unrelated healthy controls. **B)** -1 PRF levels in LCLs analyzed in **(A)**. **C)** UAA stop codon read through levels in LCLs analyzed in **(A)**.