

## **SUPPLEMENTAL MATERIALS AND METHODS**

### **Colony formation assay**

Isolation of human CD34<sup>+</sup> cells from healthy donors (n=6, AllCells, Alameda, CA) was performed using MicroBead Kit (Miltenyi, Bergisch Gladbach, Germany) following the manufacturer's instructions. For colony formation assays of human cells, BM CD34<sup>+</sup> cells were seeded at  $0.2-1 \times 10^4$  cells/mL in 12-well culture dishes with Methocult GF H4434 (STEMCELL Technologies, Vancouver, Canada). For mouse cells, mouse BM LSK cells from control-treated (n=8) and TAK243-treated (n=9) mice were added to Methocult 3434 medium (STEMCELL Technologies) and plated at 300 cells/plate in 12-well culture dishes. Colonies were evaluated after 2 weeks of culture.

### **Mice and treatment**

BL6 mice were maintained in pathogen-free conditions and their care followed animal use protocols approved by The University of Texas MD Anderson Cancer Center Institutional Care and Use Committee and US Department of Health and Human Services guidelines. Mice received TAK243 (10 mg/kg) via intraperitoneal injection twice weekly for 2 weeks. Complete blood count analysis in control-treated (n=4) and TAK243-treated (n=4) mice was performed using a HORIBA ABX Pentra analyzer (HORIBA Instruments Incorporated, Irvine, CA). Cell suspensions of BM or spleen of control-treated (n=8) and TAK243-treated (n=9) mice were incubated with antibodies and analyzed using a Gollios flow cytometer (Beckman Coulter, Indianapolis, IN). Data were analyzed using the Kaluza flow cytometry analysis software (Beckman Coulter). Mouse BM LSK cells were added to Methocult 3434 medium (STEMCELL Technologies) and plated at 300 cells/plate in 12-well culture dishes for colony formation assays.

### **Competitive transplantation assay**

CD45.2 BM LSK cells from BL6 control-treated (n=4) and TAK243-treated (n=7) mice were isolated and  $1 \times 10^6$  cells were mixed with an equal number of CD45.1 competing BM cells from wild-type mice. Cell mixtures were transplanted into lethally irradiated CD45.1 recipient mice at 8 weeks of age. BM chimerism after transplantation was assessed by FACS. Cells were stained with PE-CD45.1 and FITC-CD45.2 antibodies (BD Biosciences, San Jose, CA).

## SUPPLEMENTAL DATA

### Supplemental Figure Legend

**Supplemental Figure 1. UBA1 in human MDS. (A)** Western blot of UBA1a and UBA1b protein expression levels as well as quantitation (relative ratios of UBA1/  $\beta$ -Actin) in whole bone marrow mononuclear cells from HD and MDS showing UBA1 RNA downregulation (MDS #1 and #2) or normal UBA1 RNA expression (MDS #3). **(B-C)** Clinical characteristics groups that are significantly different between patients with and without downregulated UBA1 RNA in the MDS-EB **(B)** and MDS-LB **(C)**. P-values were calculated using one tail t-test. (NS, not significant; CG, cytogenetic characteristics; WBC, white blood cell count; ANC, absolute neutrophil count.) **(D)** A trend of increased IFN- $\beta$  cytokine levels in bone marrow plasma of HD (n=5) and patients with MDS-LB with normal UBA1 (n=6) and UBA1 downregulation (n=9). Bars represent the means + S.E.M. P-values were calculated using one tail t-test.

### Supplemental Figure 2. Study mechanisms underlying the co-occurrence of UBA1

**downregulation and SF3B1 mutations. (A)** Junction counts analysis detected significant alternative pre-mRNA splicing in bone marrow CD34+ cells that specifically occurred in patients with myelodysplastic syndrome with low blasts (MDS-LB) and *SF3B1* mutations but not in MDS-LB patients who had wild-type *SF3B1*. **(B)** Increased FOXO3 and FOXO4 genes in HD (n=12) MDS-LB with UBA1 downregulation and *SF3B1* mutations (n=6) and other MDS-LB (n=24). Bars represent the means + S.E.M. P-values were calculated using one tail t-test. **(C)** Increased

PB IFN- $\beta$  detected by ELISA in HD (n=5) MDS-LB with UBA1 downregulation and SF3B1 mutations (n=5) and other MDS-LB (n=11). Bars represent the means + S.E.M. P-values were calculated using one tail t-test. A3SS: alternative 3' splice site; A5SS: alternative 5' splice site; MXE: mutually exclusive exons; RI: retained introns, and SE: skipped exons.

**Supplemental Figure 3. TAK243 treatment in human BM HSPC and mice. (A)** Western blot of Poly-ubiquitin levels in K562 cells with TAK243 or vehicle control. **(B)** Western blot of ubiquitin and Uba1 levels in spleen cells of BL6 mice treated with TAK243 or vehicle control. **(C-G)** Impact of TAK243 treatment in PB lymphocyte count **(C)**, monocyte count **(D)**, neutrophil count **(E)** (n=4 for control and TAK243 treated mice respectively), frequency of BM Gr1+/CD11b+ myeloid cells **(F)**, and frequency of B220+ lymphocytes **(G)** (n=8 for control- and TAK243-treated mice respectively). **(H)** Reads per kilobase of transcript per million mapped reads based on RNA-seq and statistical values of complement genes C1s2 and C6 in bone marrow LSK cells of TAK243- or control-treated mice. **(I)** Flow cytometry detected annexin V+/apoptotic cells in cultured human BM CD34+ cells (left, n=6 for both TAK243 and control-treated) and in BM cells from treated mice (right, n=4 for control and n=3 for TAK243 treated mice). Bars represent the means + S.E.M. P-values were calculated using one tail t-test.

**Supplemental Table 1. Patient Cohort and Clinical Characteristics**

<b>Variable</b>	<b>MDS-EB, n=29</b>	<b>MDS-LB, n=22</b>	<b>CMML, n=21</b>
Median age (range), years	70 (55-87)	71 (42-80)	74 (47-87)
Median hemoglobin (range), g/dL	9 (6.9-14.6)	9.5 (8.2-14.3)	11.2 (8.4-14.6)
Median white blood cell count (range), $\times 10^9/L$	2.5 (0.6-9.8)	4.1 (2.8-11.5)	9.4 (3.3-59.4)
Median peripheral blood monocyte count (range), $\times 10^9/L$	0.3 (0-0.71)	0.4 (0.04-1.8)	2.3 (0.8-12.6)
Median peripheral blood neutrophil count (range), $\times 10^9/L$	0.9 (0.2-2.7)	2.3 (0.6-9.4)	6 (0.8-40.4)
Median peripheral blood platelet count (range), $\times 10^9/L$	89 (22-250)	133 (12-331)	82 (16-201)
Median bone marrow blast percentage (range)	9 (5-18)	2 (0-4)	5 (1-17)
Cytogenetic characteristics			
Diploid, no. (%)	15 (52)	14 (64)	13 (62)
Intermediate, no. (%)	4 (14)	6 (27)	5 (24)
Complex, no. (%)	10 (34)	2 (9)	3 (14)

MDS-EB, myelodysplastic syndrome with excess blasts; MDS-LB, myelodysplastic syndrome with low blasts; CMML, chronic myelomonocytic leukemia

**Supplemental Table 2. Genes upregulated in BM LSK of mice treated by TAK243 compared to mice treated by**

Gene	log2FoldCI	pvalue	padj
Igkv4-80	7.922424	4.58E-07	0.000271
Vmn1r217	7.829917	6.83E-05	0.010051
Gm3616	7.70426	0.000104	0.013302
Gm3683	7.70426	0.000104	0.013302
Syt16	7.625788	2.77E-05	0.005618
Gm30551	7.580929	0.000289	0.025531
Gm49306	7.470928	0.00034	0.028214
Trim40	7.438337	0.000437	0.031943
Ighv14-3	7.407871	3.72E-09	4.40E-06
Gm11756	7.257085	0.00053	0.035231
AC140365.1	7.250976	0.000298	0.02598
Gm5405	7.247101	0.000602	0.037118
Myh3	7.24618	1.58E-05	0.003715
6430710C18Rik	7.23201	0.000463	0.033191
Gm42827	7.221349	0.000687	0.039863
Gm50332	7.184552	0.000399	0.030366
Igkv4-57-1	7.146376	0.000581	0.036718
Gm18577	7.114389	0.000112	0.013891
Mgat5b	7.109416	8.27E-05	0.011422
Tmco5	7.101721	0.000593	0.036915
Ccdc85a	7.087252	0.00035	0.028447
Gm37779	7.082411	0.00095	0.048833
Tmprss11b	7.045858	0.000135	0.015803
Cdcp3	7.034939	0.000176	0.018359
Rp111	7.028387	0.000359	0.028727
Gm10354	7.025601	0.000203	0.02014
Adgrb3	7.017483	0.000165	0.017706
Vmn1r49	6.970209	0.000364	0.028943
Cyp3a11	6.961869	0.000596	0.036915
Gm21065	6.925577	0.000729	0.041867
Gm20853	6.925577	0.000729	0.041867
Trpv1	6.896086	0.0002	0.019996
Gm12724	6.893327	0.000904	0.0474
Gm21388	6.884426	0.000278	0.025166
Gm10832	6.855578	0.000166	0.017706
Gm6176	6.855232	0.00033	0.027925
Gm21035	6.853421	0.00054	0.03535
Fcrl6	6.84288	0.000618	0.037624
Gm21034	6.815486	0.000621	0.037701
Ighv1-5	6.774658	5.25E-07	0.000293
Gm3508	6.768522	0.000174	0.018208
9830107B12Rik	6.757542	2.53E-05	0.005279
Cyp4a30b	6.716421	0.000324	0.027602
C6	6.686207	0.000922	0.047812
Vmn1r73	6.669129	0.000551	0.035852

Gm35439	6.618838	8.82E-05	0.011918
Gm3047	6.578817	0.000192	0.019517
Gm2366	6.578323	0.000479	0.033638
Gm39043	6.565741	0.000498	0.033954
Gm26630	6.551469	0.000489	0.033851
Gm26650	6.471591	0.000162	0.017663
Gm4454	6.465396	0.000592	0.036915
Col6a5	6.455961	0.00056	0.035943
Gm7945	6.445946	0.000763	0.043095
Gm29187	6.445504	4.58E-05	0.007879
Hnr	6.439507	4.17E-05	0.007364
Gm8159	6.438589	0.000341	0.028214
Gm31577	6.393192	0.000217	0.021136
Ehf	6.381118	0.000749	0.042662
Gm7556	6.378083	0.000343	0.028214
Trim31	6.360297	0.000668	0.039576
D130079A08Rik	6.339459	0.000854	0.046184
Gm44992	6.339113	0.000298	0.02598
Gm49593	6.323357	0.000537	0.035231
Gm13346	6.233384	0.000124	0.014702
Gm6902	6.22643	0.000485	0.033851
Emx2os	6.19961	0.000186	0.01924
Gm44930	6.192322	0.000365	0.028948
Gm2117	6.183155	0.000947	0.048732
Pigr	6.140326	0.000534	0.035231
Ngef	6.124128	0.000654	0.039241
1700007P06Rik	6.102084	0.000421	0.031365
Hgd	6.022706	0.000595	0.036915
Gm6729	5.994717	2.61E-05	0.005401
Adgrb1	5.912586	0.000141	0.016243
Gm2244	5.901678	0.000582	0.036718
1700013F07Rik	5.888945	0.000793	0.044001
Gm3669	5.715371	0.000528	0.035231
Gm2101	5.698038	0.000532	0.035231
Gm2165	5.698038	0.000532	0.035231
C1s2	5.690084	0.000655	0.039241
Slc17a3	5.564693	0.000412	0.031017
Dclk1	5.462918	9.99E-05	0.012904
Six3	5.460529	0.000978	0.049439
Dnah5	5.450272	9.99E-05	0.012904
Vmn2r79	5.417947	0.000141	0.016243
Gm13415	5.33742	0.0009	0.0474
Olf1183	5.292117	0.000367	0.029036
Csmd1	5.284556	6.26E-05	0.009611
Asb15	5.109857	0.000155	0.017365
Gm3348	5.088981	0.000904	0.0474
Gm3170	5.088981	0.000904	0.0474

Pappa2	4.946791	0.000773	0.043387
A2m	4.924142	0.000982	0.049576
4931429L15Rik	4.896868	0.000811	0.044603
Gm47409	4.896502	0.000284	0.025384
Vmn1r55	4.882049	0.000349	0.028447
Gm19459	4.754927	0.000181	0.018806
1700110I01Rik	4.736556	5.04E-05	0.008289
C230057M02Rik	4.731109	0.000186	0.01924
C5ar1	4.652619	0.000565	0.036106
Ppp2r2c	4.615458	0.000763	0.043095
Scn3a	4.607301	0.000581	0.036718
Cdh10	4.599595	5.37E-05	0.008774
Fam81a	4.50421	0.000553	0.035852
Gm37087	4.492128	0.000392	0.03009
Tcp10c	4.453908	2.88E-05	0.005678
Parva	4.440782	0.000862	0.046386
Myom2	4.40196	0.00069	0.039942
Duxf3	4.380135	0.000288	0.025531
Myh6	4.291068	7.43E-05	0.010616
Gm45159	4.204214	0.000799	0.04428
Ntrk2	4.196494	0.000866	0.046489
Edil3	4.144189	0.000687	0.039863
Abcc8	3.981785	0.000964	0.049162
Nanog	3.925054	0.000791	0.044001
Rpl19-ps3	3.902462	0.000844	0.045875
Adamts1	3.822247	0.000451	0.032625
Nlrp12	3.794535	0.000212	0.020829
Gm37985	3.701192	4.64E-05	0.007926
Gm15795	3.584363	0.000237	0.022341
Fst	3.541167	0.00061	0.037342
Gm12642	3.540674	7.25E-05	0.010567
Vwa3a	3.521964	0.000437	0.031943
Gm45660	3.51337	0.000116	0.014083
Gm44792	3.288713	7.47E-05	0.010616
4930429P21Rik	3.164075	0.000535	0.035231
Gm9407	3.134437	0.000468	0.033231
Gm8341	2.875537	0.000684	0.039863
Gm49391	2.840022	0.000419	0.03132
Gm7823	2.799998	0.000746	0.042547
Gm5910	2.760416	0.000439	0.03195
Gm5511	2.735553	0.000687	0.039863
Gm8874	2.714718	8.93E-05	0.011918
Actb-ps1	2.660724	0.000586	0.036736
Gm8652	2.632289	9.90E-05	0.012904
Gm5461	2.630657	0.000159	0.017627
Gm7059	2.605758	0.000292	0.025713
Gm6192	2.580159	0.000764	0.043095

Gm37017	2.509731	0.00078	0.043609
Gm9432	2.461501	0.00016	0.017641
Gm8920	2.438561	2.28E-05	0.004817
Gm6758	2.375312	0.000858	0.04627
Gm6681	2.368504	0.000883	0.046874
Gm21960	2.31152	0.000173	0.018208
Gm671	2.297118	0.000904	0.0474
Gm16216	2.289973	0.000289	0.025531
Gm46522	2.277413	0.000769	0.043258
Ahnak2	2.262061	1.67E-05	0.003888
Synb	2.250283	0.000153	0.017225
Pkd1l2	2.176603	3.33E-05	0.006306
Gm27179	2.174965	0.000624	0.037701
Rps12-ps17	2.159689	0.000508	0.034523
Adgrg6	2.157248	0.000381	0.029746
Gm7413	2.08507	0.000488	0.033851
Gm22571	2.057904	0.00081	0.044603
Gm12430	1.994902	0.00024	0.022543
Itgb6	1.951379	0.000584	0.036736
Gm26532	1.948967	0.000792	0.044001
Gm7232	1.889665	0.000889	0.047147
Gm5529	1.852062	0.000416	0.031198
Gm5913	1.829664	0.000676	0.039863
Synpo	1.82954	5.85E-06	0.00189
Gm35853	1.812366	1.51E-14	8.55E-11
Gm37352	1.75583	0.000441	0.031985
Pi16	1.694409	1.37E-05	0.003425
Gm49739	1.65843	0.000685	0.039863
Gm8922	1.65702	0.000973	0.049382
Sag	1.619341	0.000109	0.013661
St8sia6	1.577034	4.56E-10	7.62E-07
Igkv1-110	1.454589	0.000351	0.028447
Slc4a8	1.431963	1.99E-06	0.000775
Gm4247	1.414173	5.27E-06	0.001721
Amd-ps4	1.400147	0.000496	0.033954
Gm40645	1.399946	2.23E-06	0.000846
Pdcd1lg2	1.39353	1.02E-08	9.31E-06
Hsf2bp	1.390347	1.39E-05	0.003425
Mgam	1.385155	0.000496	0.033954
Gm43145	1.344091	0.000753	0.04279
Krt80	1.274184	3.44E-10	6.11E-07
Tlr12	1.269389	5.78E-05	0.009231
Ryr1	1.180893	1.68E-06	0.000696
Gm47547	1.159535	0.000678	0.039863
Car12	1.129994	0.000284	0.025384
Rab44	1.125375	9.80E-07	0.000488
Rab11fip4	1.120982	0.000685	0.039863

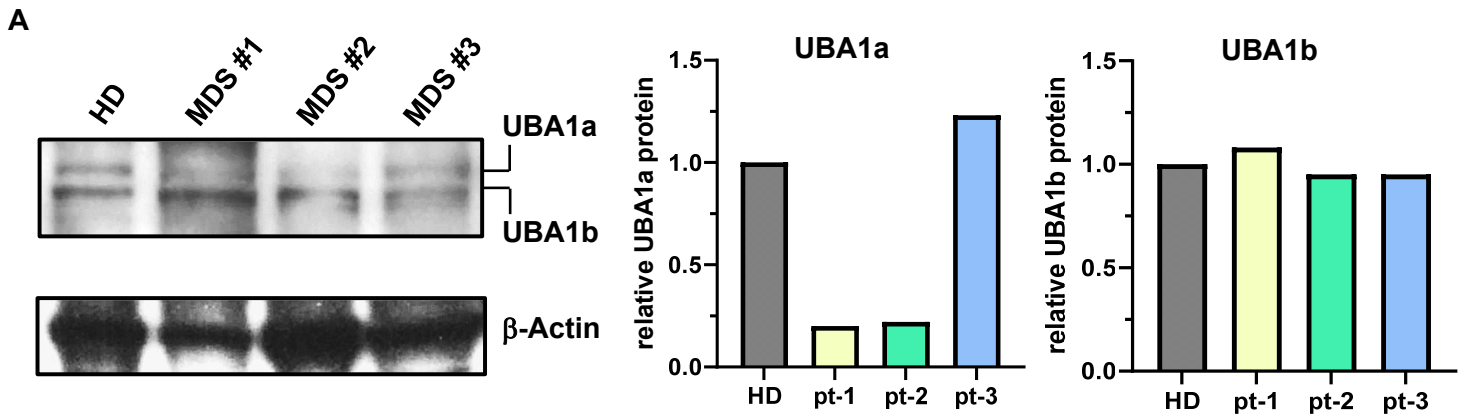


Itga2b	1.105217	2.24E-09	2.89E-06
Bpifc	1.092356	8.62E-05	0.011829
Fgf3	1.063914	0.000305	0.026318
9530018F02Rik	1.048702	1.74E-08	1.54E-05
Ahnak	1.047899	1.51E-06	0.000669
Cp	1.024253	0.000166	0.017706
Sbsn	1.012909	1.22E-05	0.003201
Gm45203	1.012082	6.03E-07	0.000323
Lag3	0.99895	6.12E-05	0.009481
B230362B09Rik	0.991767	5.05E-05	0.008289
Snord43	0.971408	0.000352	0.028447
Ttn	0.970557	7.43E-06	0.002247
Map3k6	0.965797	1.74E-06	0.000706
Gm16464	0.961741	0.000558	0.035943
Gm37747	0.958882	0.00096	0.049051
Arfgef3	0.948043	3.77E-06	0.001323
Zbtb16	0.946326	1.02E-05	0.002781
Itgam	0.941599	3.22E-06	0.001143
I730030J21Rik	0.920229	4.97E-06	0.001662
B4galnt4	0.907007	2.79E-05	0.00562
Rps10	0.90281	0.000939	0.048519
Robo3	0.90162	0.000311	0.026763
Thy1	0.896984	2.86E-05	0.005678
Emilin2	0.873467	9.41E-07	0.000477
Epop	0.871962	0.000108	0.013661
Efr3b	0.862247	6.97E-06	0.002152
Gm37069	0.861381	0.000976	0.049439
Gm43420	0.86079	0.000166	0.017706
Mmp2	0.830217	0.000583	0.036718
Dcun1d4	0.82765	6.37E-06	0.00201
Snora43	0.825452	5.55E-05	0.009019
Ffar2	0.819802	1.01E-06	0.000494
C030034L19Rik	0.817393	1.68E-07	0.000116
Epb41l4a	0.817175	0.000391	0.03009
Gm28530	0.815777	4.40E-05	0.007622
Mast1	0.796958	0.000282	0.025362
Stx3	0.791415	5.61E-07	0.000307
Gm45718	0.768664	3.03E-05	0.005931
Rrs1	0.757398	0.000498	0.033954
Soat2	0.755157	0.000487	0.033851
Gm15657	0.744337	0.000301	0.026185
Ung	0.741701	2.26E-05	0.004817
Slc18a2	0.739392	0.000126	0.014933
Snhg15	0.734861	8.23E-06	0.002386
Gm32742	0.708734	0.000515	0.034898
P2ry2	0.698658	0.000734	0.04204
Fkbp5	0.686387	7.35E-05	0.010616

Nlrc4	0.677026	0.000921	0.047812
Aatf	0.675274	0.000168	0.017841
Znhit6	0.647403	6.50E-05	0.009808
Ctps	0.647058	0.000387	0.030058
Fabp5	0.6444	0.000481	0.033658
Lilr4b	0.644067	0.00032	0.027483
Timm10	0.641554	0.000386	0.030058
Dancr	0.632353	0.000245	0.022913
Mdn1	0.628068	4.68E-05	0.007926
Rrp15	0.625446	0.000684	0.039863
Cd244a	0.624188	1.82E-05	0.004098
5430416N02Rik	0.622615	8.91E-06	0.002531
Pus7	0.618376	8.87E-05	0.011918
Gpatch4	0.616256	0.000565	0.036106
D030028A08Rik	0.616163	0.000988	0.049694
Pwp2	0.615213	4.23E-05	0.007428
Baz1a	0.614372	0.000476	0.033546
Rec8	0.609234	3.33E-05	0.006306
Mboat1	0.606005	0.000705	0.040698
Ftsj3	0.60432	0.000432	0.031796
Pik3cb	0.60306	0.000532	0.035231
Sntb2	0.60163	0.000425	0.031524
Lat2	0.583476	3.27E-05	0.006273
Trmo	0.574866	0.000294	0.025804
Pinx1	0.571942	0.000623	0.037701
Mettl1	0.564658	0.000424	0.031524
Firre	0.563546	8.30E-07	0.000429
Pdgfrb	0.56185	0.0002	0.019996
Trf	0.553612	0.000392	0.03009
Prodh	0.550239	1.47E-05	0.00354
Nol10	0.545558	0.000842	0.045875
Akap1	0.543177	9.97E-05	0.012904
Chn2	0.542024	9.33E-05	0.012337
Svil	0.532524	1.04E-05	0.002781
Mrto4	0.515741	0.000855	0.046184
Pprc1	0.506313	1.47E-05	0.00354
Tsr1	0.506174	3.70E-07	0.000234

y vehicle controls.

Supplemental Figure 1



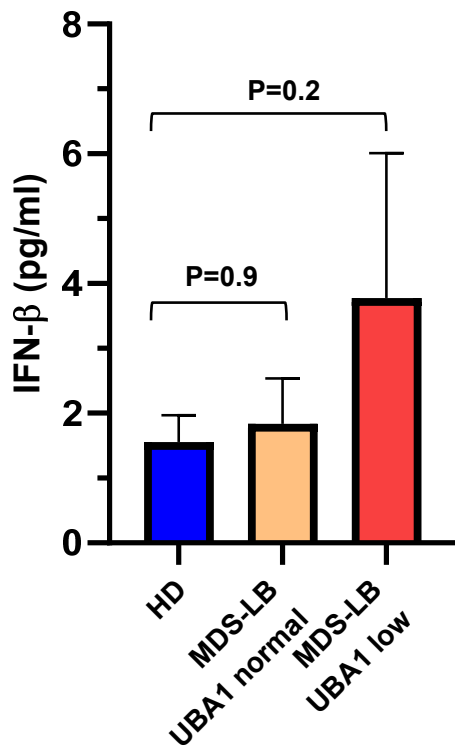
**B**

MDS-EB	Complex CG	WBC ( $10^9/L$ )	ANC ( $10^9/L$ )
UBA1 low (n=11)	55%	2.2	0.69
UBA1 normal (n=18)	22%	3.5	1.5
P-value	0.05	0.02	0.003

**C**

MDS-LB	Hemoglobin (g/dL)	Platelets ( $10^9/L$ )
UBA1 low (n=11)	9.2	187
UBA1 normal (n=11)	10.8	105
P-value	0.048	0.02

**D**

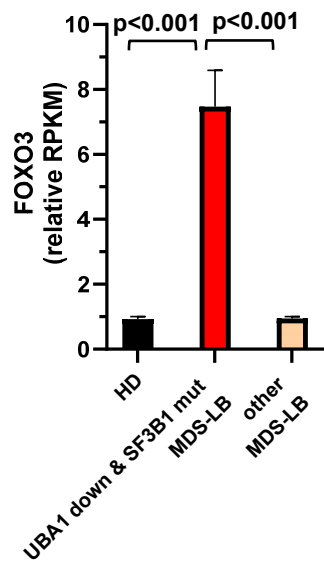


## Supplemental Figure 2

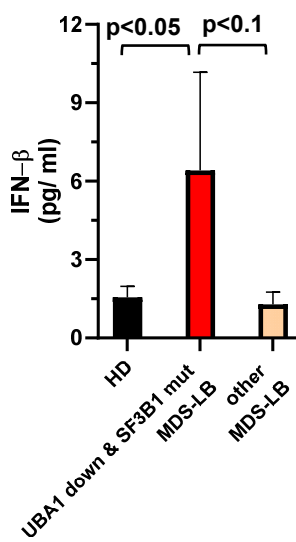
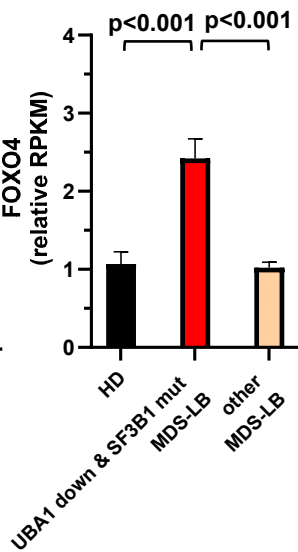
A

<b><i>SF3B1</i> mutation-specific alternative splicing events</b>	
A3SS_JCEC	293
A5SS_JCEC	285
MXE_JCEC	45
RI_JCEC	29
SE_JCEC	408
<b>Total</b>	<b>1060</b>

B

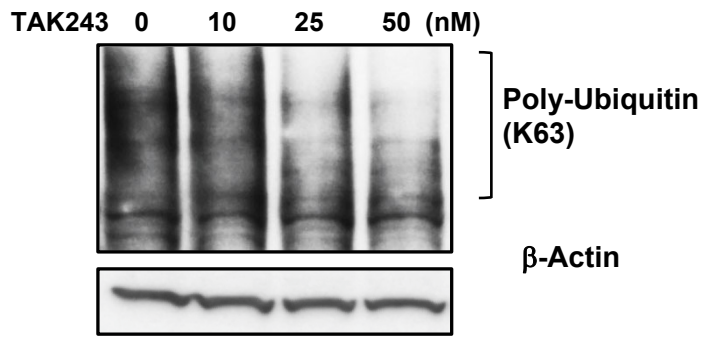


C

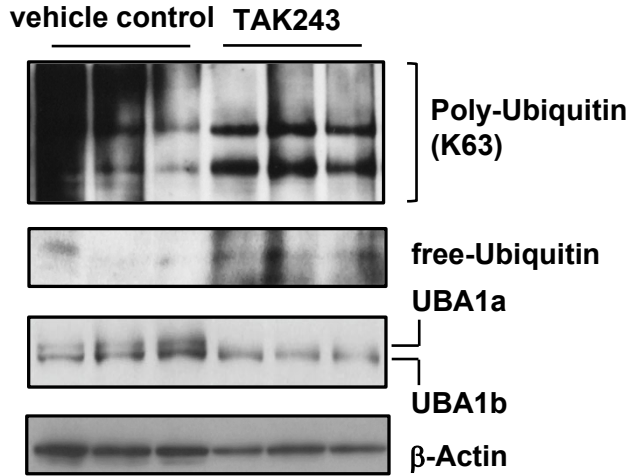


Supplemental Figure 3

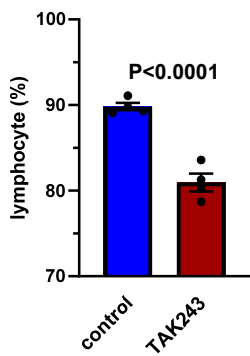
A



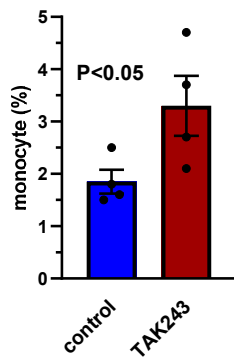
B



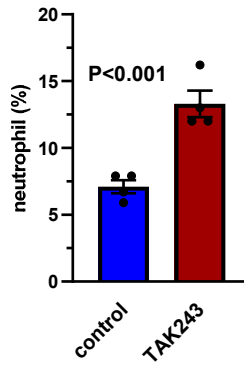
C



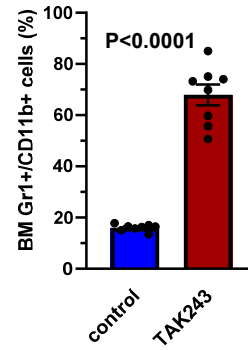
D



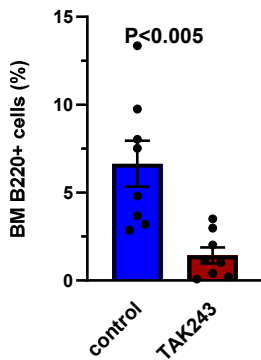
E



F



G



H

Gene	C1s2	C6
Base mean	10.77	7.71
Log <sub>2</sub> fold change (TAK243 vs ctrl)	5.69	6.69
P	0.0007	0.0009
Adjusted P	0.039	0.048

I

