# 生殖細胞におけるIKZF1遺伝子変異と小児ALL（acute lymphoblastic leukemia）に対するリスク

聖路加国際病院
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山原直紀

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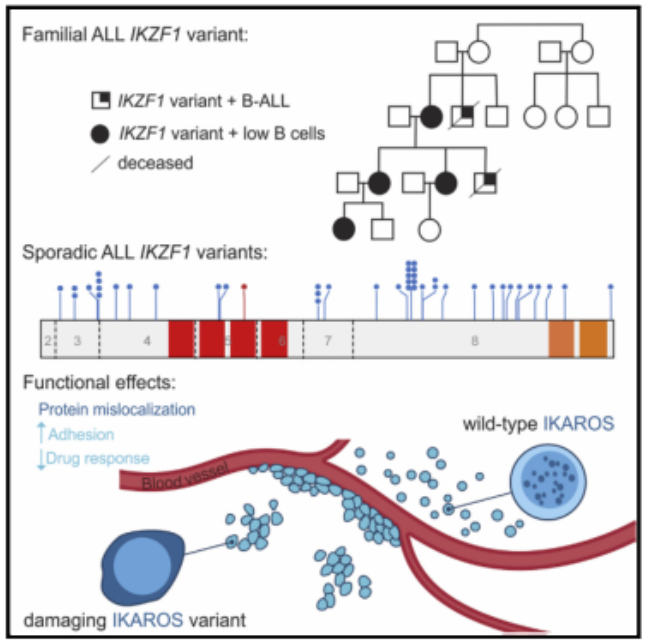
Article

Cancer Cell

# Germline Genetic *IKZF1* Variation and Predisposition to Childhood Acute Lymphoblastic Leukemia

## Graphical Abstract

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## In Brief

Churchman et al. identify 28 unique germline *IKZF1* coding variants in 45 children with acute lymphoblastic leukemia. Many of these variants are not predicted to be damaging using *in silico* prediction tools, but functional tests reveal that the majority of them have deleterious effects on IKAROS function.

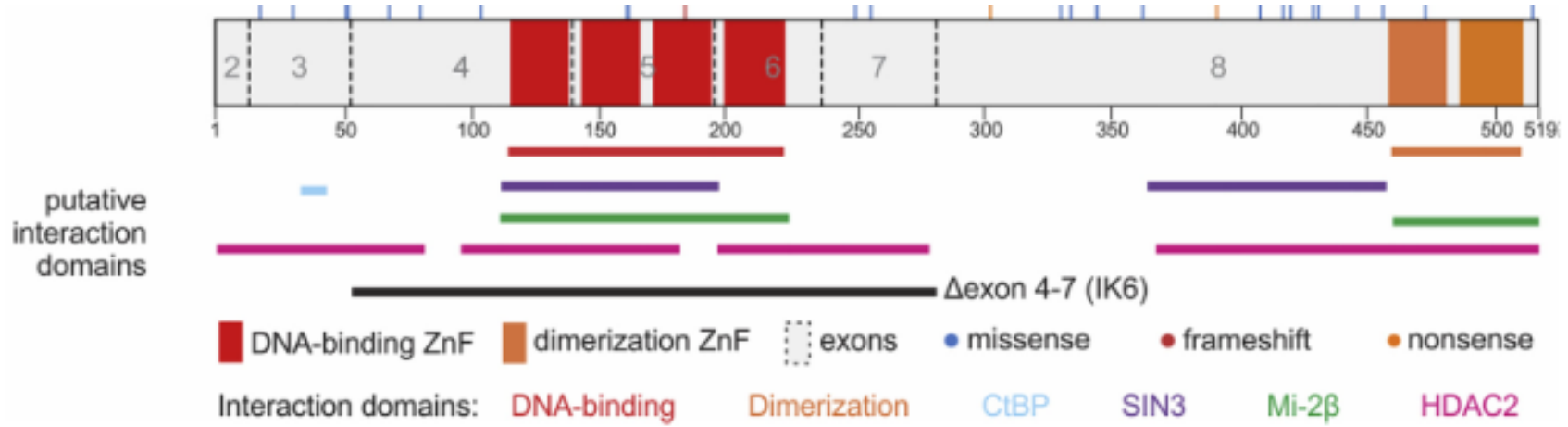
# 背景

- 小児ALLの治癒率は90%に達するが、いまだに小児の死亡の主要な原因である
- IKZF1の欠失および変異は小児ALLの予後不良因子である
- ALL発症に遺伝的素因が関わるとするエビデンスが構築されつつある

背景

# IKZF1遺伝子、IKAROSとは

- IKZF1はIKAROSをコードする遺伝子
- IKAROSは4つのN末端側のzinc fingerと、2つのC末端側のzinc fingerを持ち、それぞれDNA結合と二量体化に関わる
- その他、SIN3などの転写に関わる蛋白質と相互作用する部位がある



# 背景
# 生殖細胞におけるIKZF1変異

- 近年、遺伝性低γグロブリン血症における IKZF1の生殖細胞変異が報告されている
- さらに、IKZF1の生殖細胞変異を認める免疫不全の患者29例のうち、2例にALLが報告されている
- 著者らはまず、小児ALLにおいてIKZF1の生殖細胞変異を有する割合を調査することとした

# 方法・結果

# 生殖細胞におけるIKZF1変異の同定

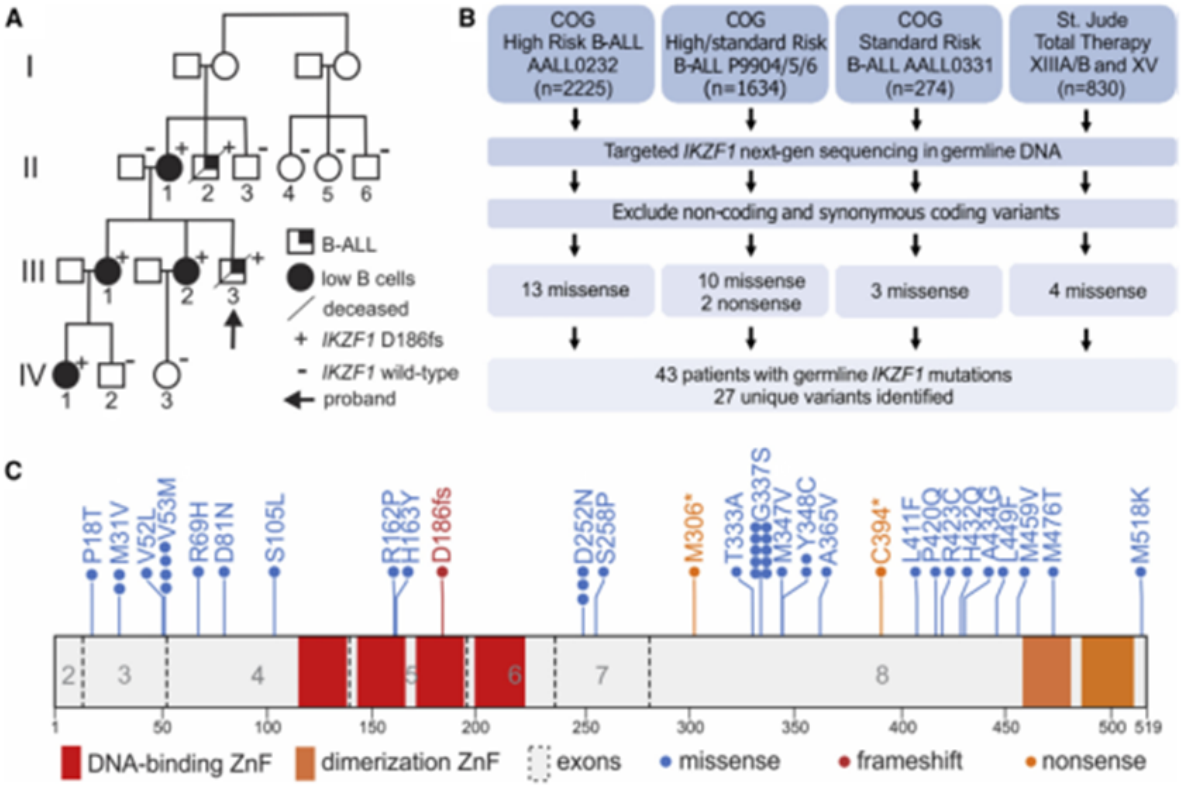



**Figure 1. Germline *IKZF1* Variants in Pediatric ALL Patients**

(A) Pedigree of the index family demonstrating a germline *IKZF1* variant (c.del556; p.D186fs) with incomplete penetrance of ALL. An arrow denotes the proband, squares and circles represent males and females, respectively, inset black boxes represent individuals with B-ALL, filled circles indicate low B cell numbers, "+" identifies individuals harboring the heterozygous germline *IKZF1* D186fs allele, "–" identifies individuals with wild-type *IKZF1* alleles, and slashes indicate deceased family members.

(B) CONSORT diagram of St. Jude Children's Research Hospital and Children's Oncology Group ALL patients included in this study.

(C) Protein domain plot of IKAROS and the amino acid substitutions predicted to result from the germline *IKZF1* variants identified in this study.

See also Figure S1 and Tables S1–S4.

方法・結果

# 生殖細胞におけるIKZF1変異の同定

- IKAROSのエキソン領域における28の変異を同定した
- D186fs（フレームシフト変異）
  - ドイツのChildren’s University Hospitalで認められた家族性B-ALL患者および血縁者から同定
- 27のミスセンス変異およびナンセンス変異
  - 4963人を対象としたSt. Jude Children’s Research HospitalとChildren’s Oncology Groupによる臨床研究で、47人から同定

# 方法･結果

# IKZF1変異の機能的影響①

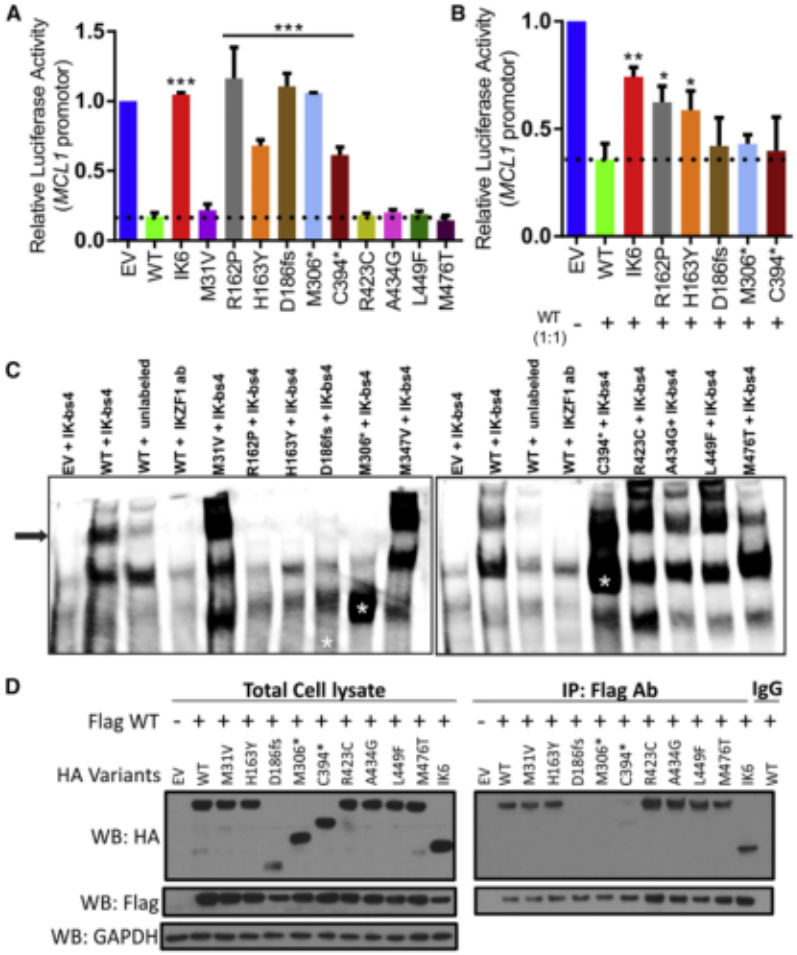



**Figure 2. Effects of *IKZF1* Variants on Transcriptional Repression, Dimerization, DNA Binding, and Nuclear Localization**

(A) Relative *MCL1* promoter activity in luciferase reporter assays using HEK293T cells ectopically expressing IKAROS variants compared with wild-type (WT), empty vector (EV), and dominant-negative IK6 as controls.

(B) Relative *MCL1* promoter activity after wild-type IKAROS is co-expressed 1:1 with the subset of IKAROS variants that lose the ability to transcriptionally repress *MCL1*.

(C) Electrophoretic shift assays performed using nuclear extracts of HEK293T cells ectopically expressing wild-type or variant IKAROS. Extracts were allowed to bind biotin-labeled probes containing a consensus IKAROS binding site (IK-bs4); as controls, excess unlabeled probe and IKAROS antibody were used as binding competitors. Arrow indicates full-length IKAROS-containing complexes and asterisks indicate truncated size of nonsense variants.

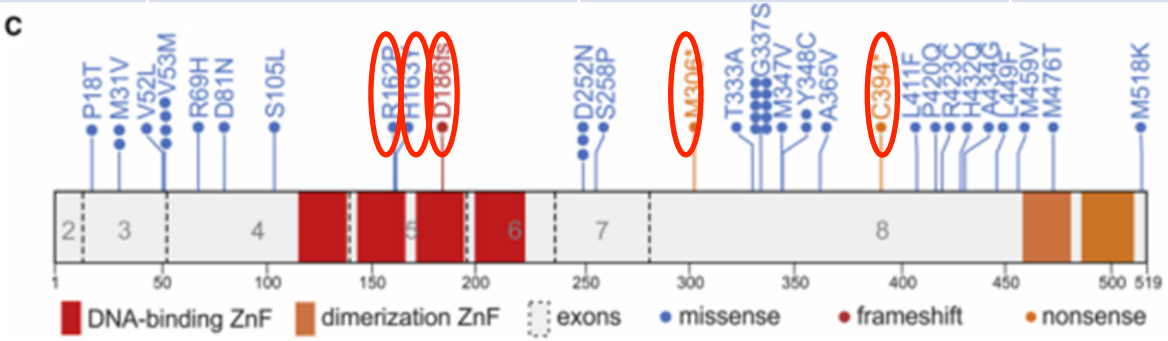
(D) Co-immunoprecipitation (IP) of FLAG-tagged wild-type and HA-tagged wild-type or variant IKAROS in HEK293T cells to demonstrate the ability of each variant to dimerize.

Data in (A) and (B) are presented as mean ± SD of three technical replicates; Student's t test compared with wild-type, ***$p < 0.0005$, **$p < 0.005$, *$p < 0.05$. See also Figure S3.

# 方法･結果

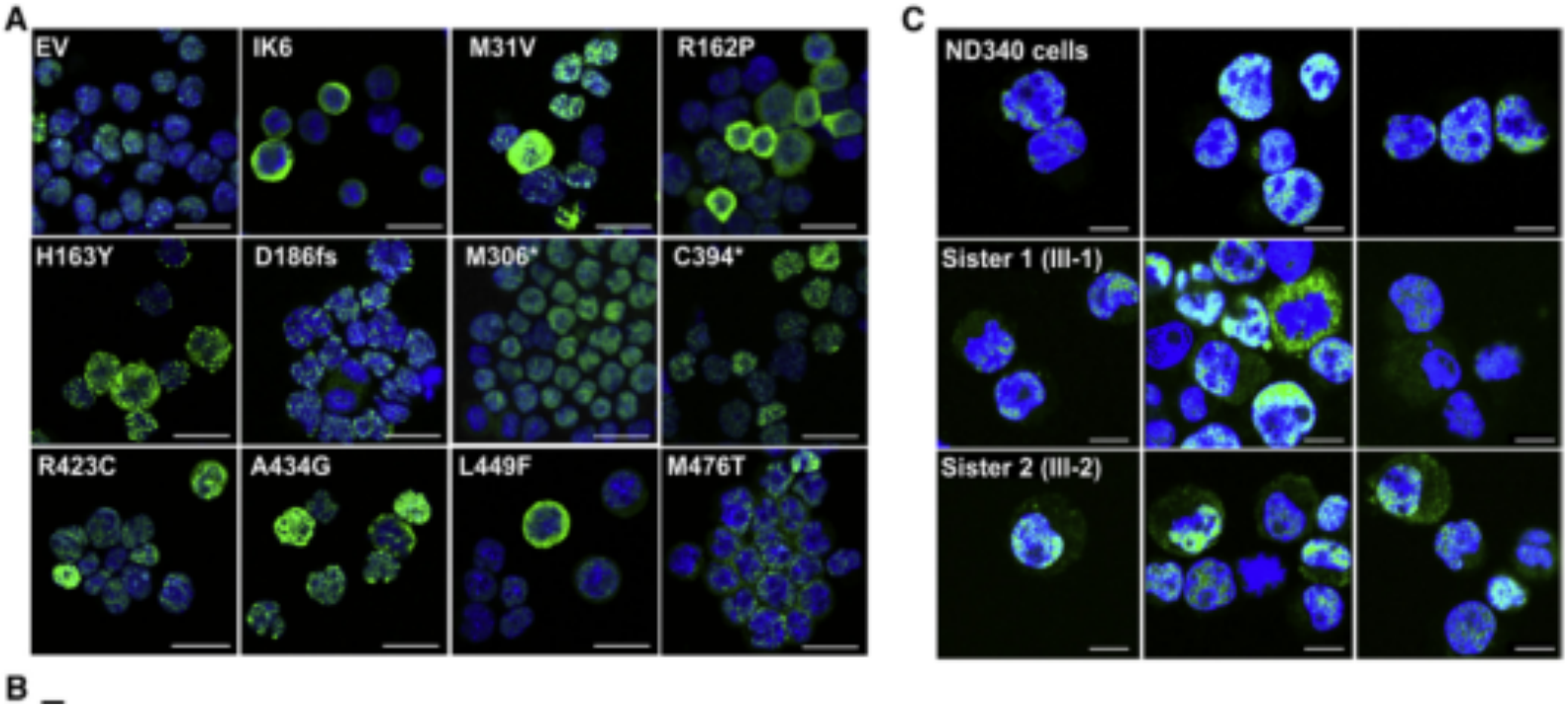
# IKZF1変異の機能的影響①

| MCL1抑制活性を低下させた変異 | 変異の様式 | DNA結合の障害 | 二量体化の障害 |
|---|---|---|---|
| R162P | Missense | ○ | × |
| H163Y | Missense | ○ | × |
| D186fs | Frameshift | ○ | ○ |
| M306 | Nonsense | × | ○ |
| C394 | Nonsense | × | ○ |



# 方法･結果

# IKZF1変異の機能的影響②



# 方法･結果

# IKZF1変異の機能的影響②

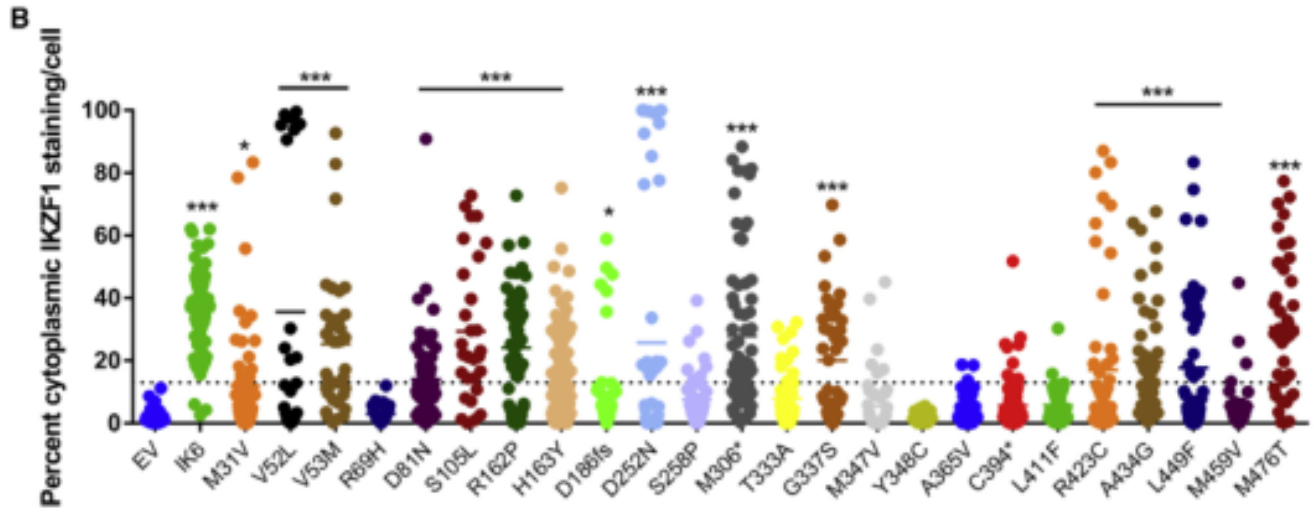



**Figure 3. Subcellular Mislocalization of IKAROS Variants**

(A) Subcellular localization of endogenous wild-type (WT) and ectopically expressed variant IKAROS (green) in primary mouse *Arf*$^{-/-}$ BCR-ABL1$^{+}$ pre-B cells detected by immunofluorescence using an antibody to the IKAROS N terminus capable of detecting all variant proteins and an AlexaFluor 488-conjugated secondary antibody. Nuclei (blue) are observed by DAPI staining.

(B) Quantification of the amount of IKAROS immunostaining found in the cytoplasm of *Arf*$^{-/-}$ BCR-ABL1$^{+}$ pre-B cells ectopically expressing each N-terminal variant. $n \geq 25$ cells; Student's t test compared with EV, ***$p < 0.0005$, *$p < 0.05$. Short horizontal lines of each corresponding color indicate the mean for each variant. The dotted horizontal line indicates the maximum percentage of cytoplasmic IKAROS staining observed in empty vector (EV) cells. All variants were tested but those that retained IKAROS activity were not evaluable as their ectopic expression resulted in cell death.

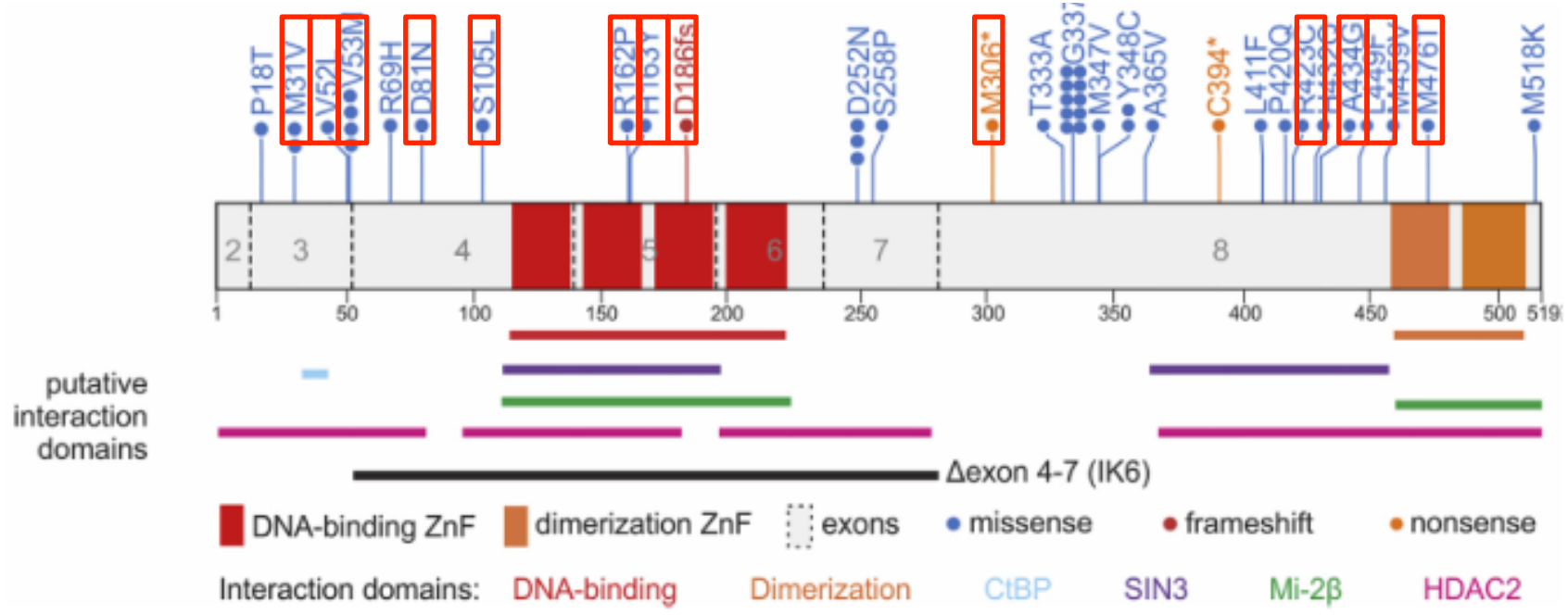
(C) Immunostaining of IKAROS in B cells isolated from individuals III-1 and III-2 in the index family shown in Figure 1 compared with ND340 cells isolated from a normal bone marrow donor harboring a wild-type *IKZF1*.

Scale bars, 20 μm (A) and 10 μm (C). See also Figure S3.

# 方法･結果

# IKZF1変異の機能的影響②

- IKAROSの細胞質への偏在を認めた変異



# 方法･結果

# IKZF1変異の機能的影響③

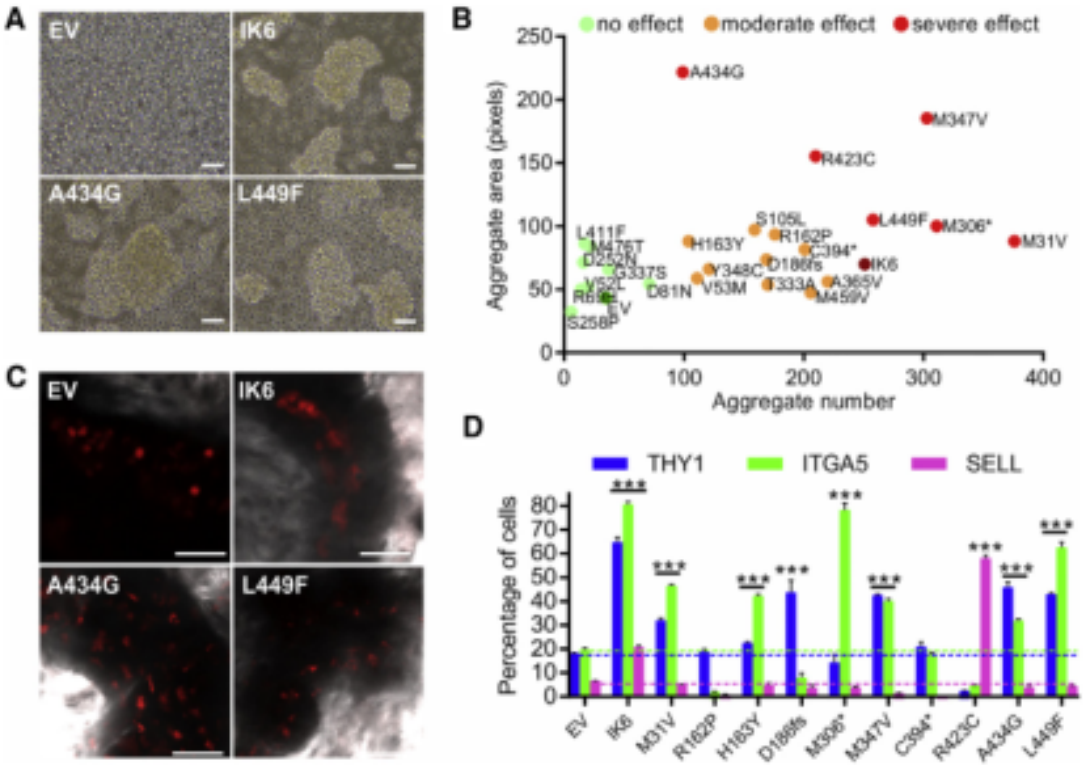



**Figure 4. Effects of *IKZF1* Variants on Cell Adhesion**

(A) Representative *in vitro* suspension cultures of primary mouse $Arf^{-/-}$ BCR-ABL1$^{+}$ pre-B cells transduced with empty vector (EV) or expressing the dominant-negative IK6 or IKAROS variants.

(B) Quantification of the number and size of cell-cell aggregate clusters in suspension cultures of $Arf^{-/-}$ BCR-ABL1$^{+}$ pre-B cells expressing IKAROS variants compared with empty vector (EV) and IK6.

(C) *Ex vivo* confocal imaging of the undisturbed bone marrow niche in calvaria of mice 3 days after inoculation with $Arf^{-/-}$ BCR-ABL1$^{+}$ pre-B cells expressing IKAROS variants.

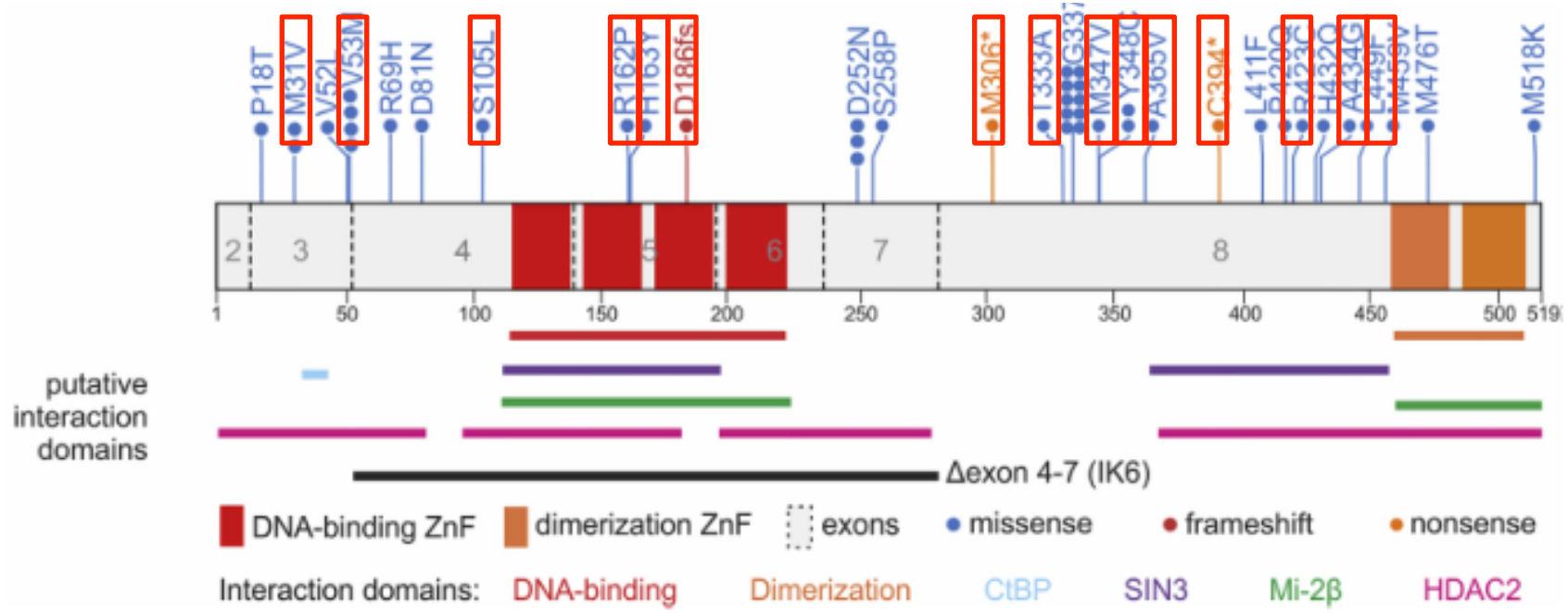
(D) Flow-cytometric analyses of $Arf^{-/-}$ BCR-ABL1$^{+}$ pre-B cells co-expressing IKAROS variants for adhesion molecules, THY1, ITGA5, and L-selectin (SELL); Data represent the mean ± SD for three technical replicates; colored dashed lines indicate mean percentage of EV cells. Student's t test compared with EV, ***$p < 0.0005$.

Scale bars, 20 μm. See also Figure S4.

# 方法･結果

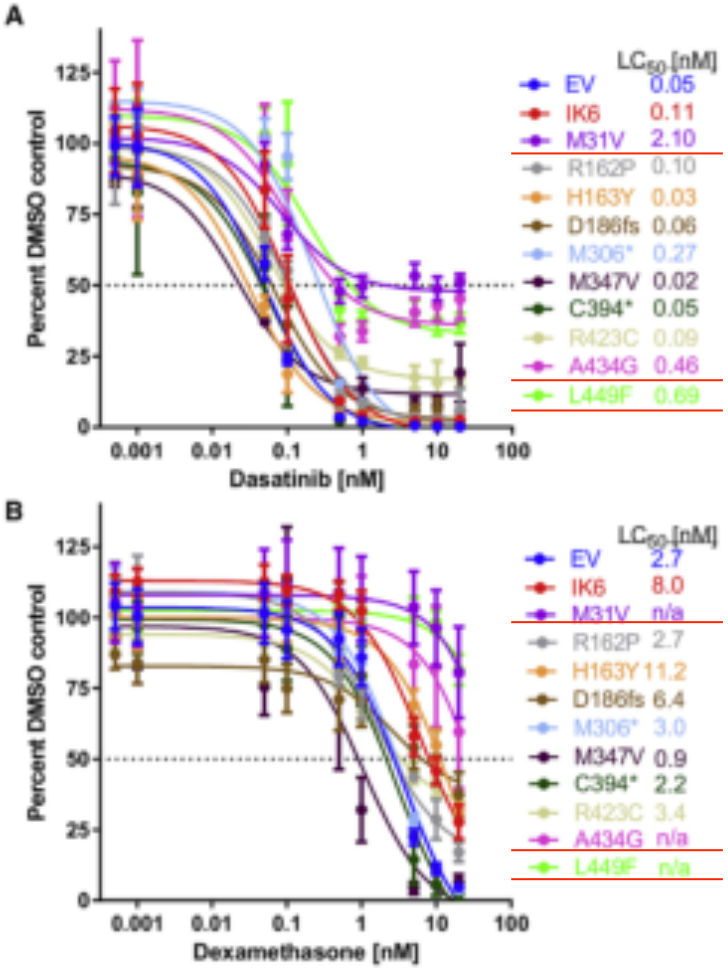
# IKZF1変異の機能的影響③

- 細胞凝集を多く認めた変異



# 方法･結果

# IKZF1変異の薬物反応性への影響



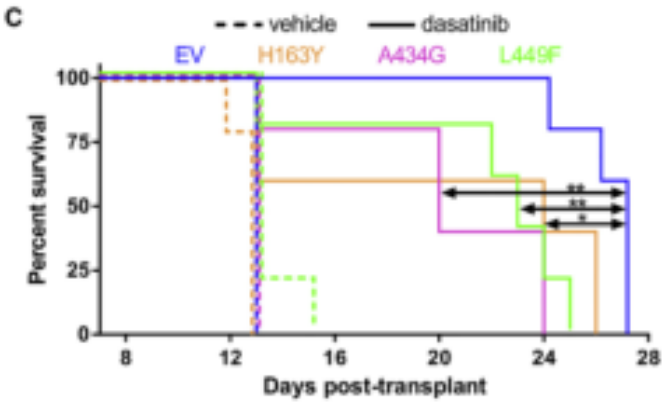




**Figure 5. Effects of *IKZF1* Variants on Sensitivity to Drugs**

(A and B) Cell viability of *Arf*$^{-/-}$ BCR-ABL1$^{+}$ pre-B cells transduced with empty vector (EV) or expressing IKAROS variants at increasing concentrations of dasatinib (A) or dexamethasone (B) after 72 hr of drug exposure *in vitro*. Data represent mean ± SD for three biological replicates performed in triplicate.

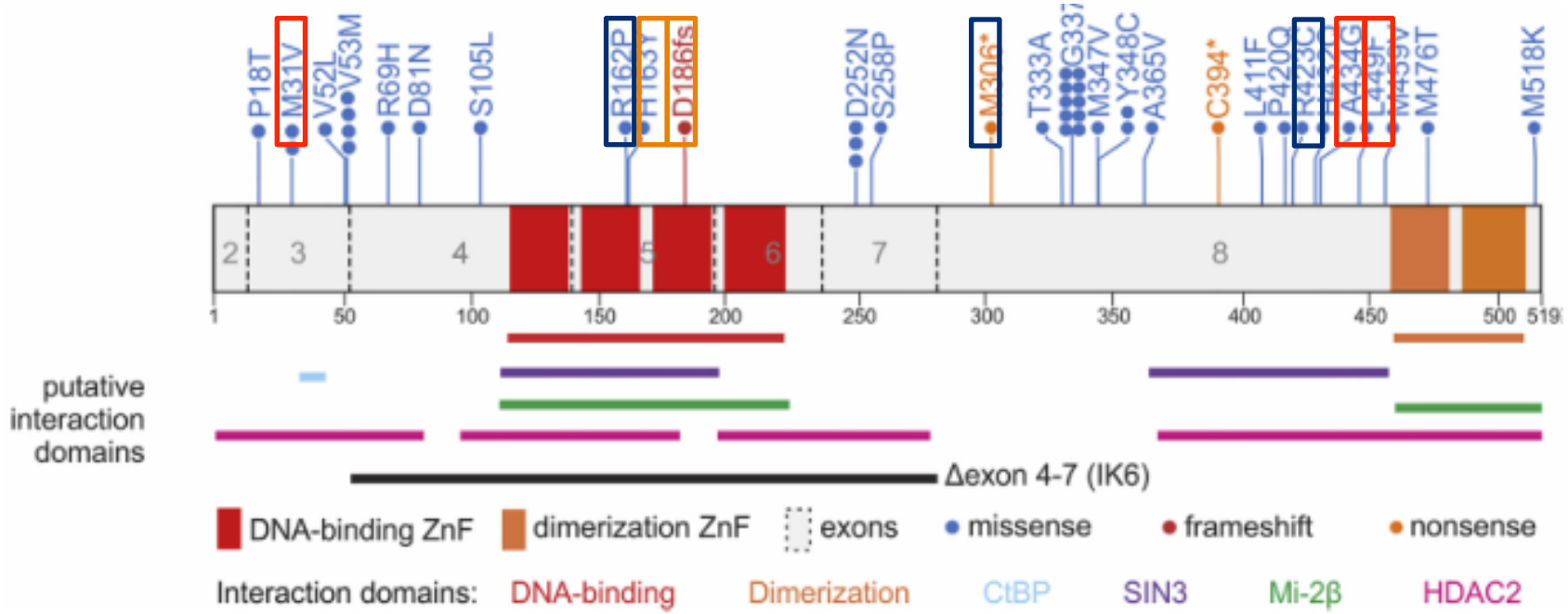
(C) Kaplan-Meier survival curve of mice inoculated by tail vein with *Arf*$^{-/-}$ BCR-ABL1$^{+}$ pre-B cells expressing either empty vector or IKAROS variants and dosed daily starting 7 days post inoculation with either vehicle or 10 mg/kg dasatinib by mouth; n = 5 mice per group; Mantel-Cox log rank compared with EV, $^{**}p < 0.005$, $^{*}p < 0.05$.

See also Table S5.

# 方法･結果

# IKZF1変異の薬物反応性への影響

- ダサチニブとデキサメタゾン両方に対する抵抗性
  - M31V、A434G、L449F
- ダサチニブにのみの抵抗性
  - R162P、M306、R423C
- デキサメタゾンにのみの抵抗性
  - H163Y、D186fs



# 方法･結果

# IKZF1変異の影響のまとめ

- 28の変異のうち22が有害なもので、6が無害なものであった
- 有害な変異、無害な変異、wild-typeのIKZF1を持つ先述の47人の患者同士の比較で、人口統計及びALLの臨床上の特徴において特記すべき差異は認めなかった

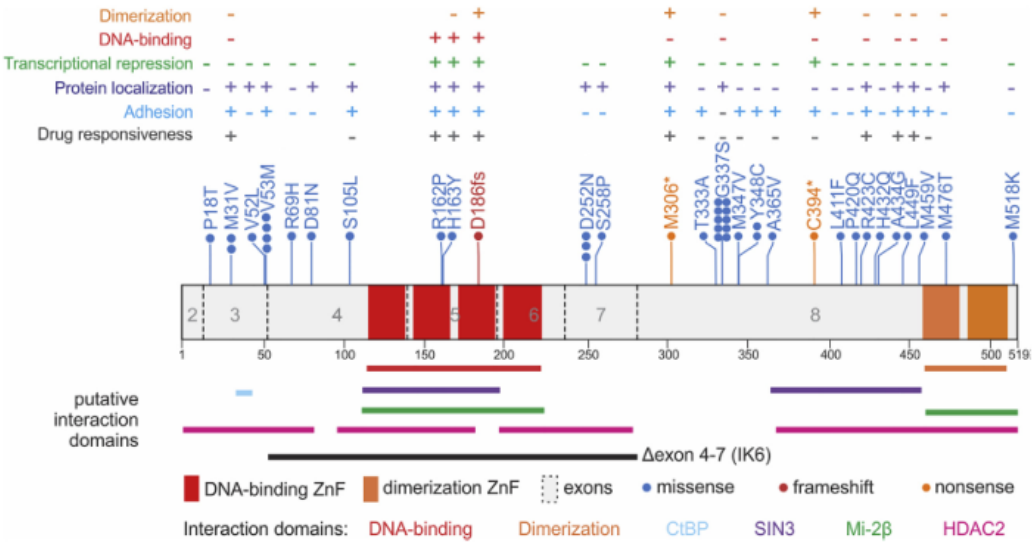



**Figure 6. Summary of Variant Effects**

The position of each variant within the protein is shown along with the consequences noted for each assay, denoted as "+" if an effect was observed, and "−" if the variant had no effect; blanks denote that the variant was not included in the assay. Putative protein interaction domains that have been previously described for mouse IKAROS are noted below the protein structure. See also Table S5.

# 考察

- ALLにおける遺伝素因となるIKZF1の生殖細胞変異を同定し、その多くの変異がIKAROSの機能を失わせる効果があることが分かった
- 家族性分類不能型免疫不全症におけるIKZF1変異はDNA結合ドメインに限られていたため、同部位が障害されるとIKAROS機能が大きく障害されると考えられていた

# 考察

- 今回著者らが発見した変異の多くがDNA結合ドメインおよび二量体化ドメインの外にあったが、28変異のうち22が有害なものであった
- これらの変異ではDNA結合能および二量体化能の低下を認めなかったため、SIN3などの結合障害が細胞内局在、細胞接着、薬物反応性の障害につながったと考えられる

# 考察

- 今回はマウスの細胞に人由来のIKZF1を形質導入したものを用いて細胞局在、細胞粘着、薬物反応性を調べているが、SIN結合部位はヒトとマウスで保存されている割合が小さく、ヒトの細胞で直接的に同部位とSINの働きを調べる研究が求められる

# 考察

- また、IKZF1の生殖細胞変異がALLのリスクとなり、かつ薬物治療への反応性に影響を及ぼすことを示した
- 家族性ALLは珍しいものであるが、その分析を通じ、疾患の罹患に関してより普遍的な役割を果たしている生殖細胞変異の同定や、その治療の結果について理解を深めることができた