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Supplemental Methods

CALR protein purification. CALR variants were cloned into the pBAD-DEST49 vector (Invitrogen) and transformed into BL21 DE3 bacterial cells (Agilent). Protein expression was induced with 0.02% arabinose, and proteins were purified using the ProBond Purification System (Invitrogen).

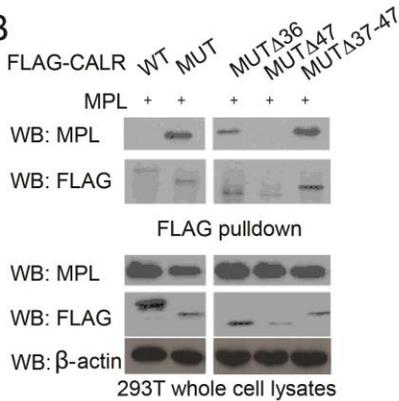
In Vitro Binding Assay. CHO-derived recombinant MPL was purchased R&D Systems. E. coli-derived recombinant ERp57 was purchased from Abcam.

Supplemental Figure 1

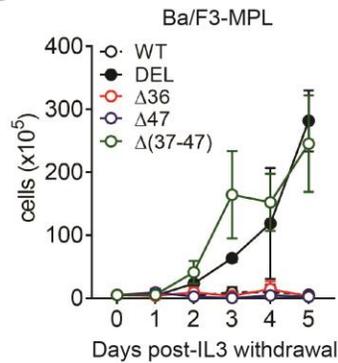
A

52 bp del QDEEQRTRRMMRTKMRMRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA
 52 bp del Δ 10 QDEEQRTRRMMRTKMRMRMRRTRRKMRRKMSPARPRTSCR
 52 bp del Δ 18 QDEEQRTRRMMRTKMRMRMRRTRRKMRRKMSP
 52 bp del Δ 28 QDEEQRTRRMMRTKMRMRMRRT
 52 bp del Δ 36 QDEEQRTRRMMRTKM
 52 bp del Δ 47 QDEE
 52 bp del Δ 37-47 QDEE _____RMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA

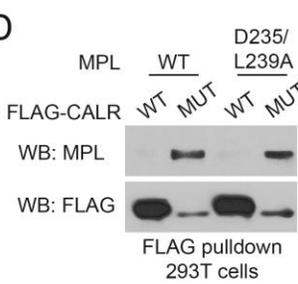
B



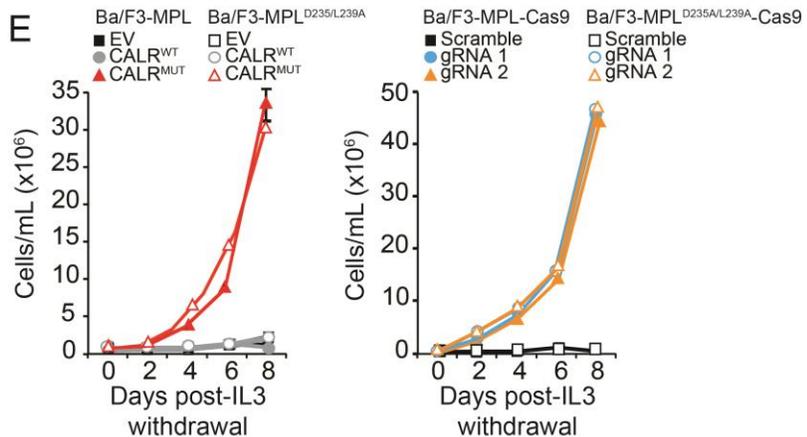
C



D



E



(A) Schema depicting serial C-terminal truncation mutants of mutant CALR. (B) Immunoblotting of FLAG immunoprecipitated proteins and whole cell lysates from 293T cells co-transfected with FLAG-CALR wild type (CALR^{WT}), FLAG-CALR 52 bp deletion (CALR^{MUT}), FLAG-CALR 52 bp deletion C-terminal truncation mutants Δ 47 or Δ 37-47 demonstrates that only deleting the 11 amino acid positively-charged stretch (QRTRRRMMRTKM) immediately adjacent to the QDEE motif shared between the wild type and mutant protein abolishes binding to MPL. (C) Growth curves in Ba/F3-MPL cells expressing CALR^{WT}, CALR^{MUT}, or CALR^{MUT} C-terminal truncation mutants Δ 47 or Δ 37-47

demonstrates that only deleting the 11 amino acid positively-charged stretch (QRTRRRMMRTKM) immediately adjacent to the QDEE motif shared between the wild type and mutant protein abolishes the transforming capacity of mutant CALR. (D) Immunoblotting of FLAG immunoprecipitated proteins from 293T cells co-expressing FLAG-CALR variants and wild type MPL or the TPO binding mutant form of MPL (D235A/L239A) demonstrates that mutant CALR can bind to MPL when the TPO binding pocket is mutated. (E) Growth curves in Ba/F3 cells stably expressing wild type or D235/L239A MPL and CALR^{WT}, CALR^{MUT}, or endogenous mutant Calr by CRISPR-Cas9 gene editing demonstrates that mutation of the TPO binding pocket of MPL does not affect that ability of MPL to support mutant CALR-mediated hematopoietic transformation.

Supplemental Table 1. Summary of phenotypes of various CALR and MPL mutations

	<i>CALR sequence</i>	<i>MPL sequence</i>	<i>cytokine-independence</i>	<i>binding</i>
<i>CALR truncations</i>	del52-D10	WT	+	+
	del52-D18	WT	+	+
	del52-D28	WT	+	+
	del52-D36	WT	-	+
	del52-D47	WT	-	-
<i>CALR point mutants</i>	del52-D135L	WT	-	-
	del52-D317A	WT	-	-
	del52-P19K/V21E	WT	+	+
	del52-W244G	WT	+	+
	del52-EEDE	WT	+	+
	del52-H153G	WT	+	+
<i>MPL tyrosine mutants</i>	del52	FYY	strong	+
	del52	YFY	weak	+
	del52	YYF	strong	+
	del52	FFY	weak	+
	del52	FYF	strong	+
	del52	YFF	weak	+
	del52	FFF	weak	+