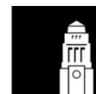

UNIVERSITY OF LEEDS

This is a repository copy of *Defining the requirements for the pathogenic interaction between mutant calreticulin and MPL in MPN*.

White Rose Research Online URL for this paper:
https://eprints.whiterose.ac.uk/125969/

Version: Supplemental Material

**Article:**

Elf, S, Abdelfattah, NS, Baral, AJ et al. (7 more authors) (2018) Defining the requirements for the pathogenic interaction between mutant calreticulin and MPL in MPN. Blood, 131 (7). pp. 782-786. ISSN 0006-4971

https://doi.org/10.1182/blood-2017-08-800896

(c) 2017, American Society of Hematology. This research was originally published in Blood Online, 'Elf, S, Abdelfattah, NS, Baral, AJ et al (2017). Defining the requirements for the pathogenic interaction between mutant calreticulin and MPL in MPN. Blood.' Published December 29, 2017. DOI: https://doi.org/10.1182/blood-2017-08-800896

**Reuse**
Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

**Takedown**
If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

eprints@whiterose.ac.uk
https://eprints.whiterose.ac.uk/

**Supplemental Methods**

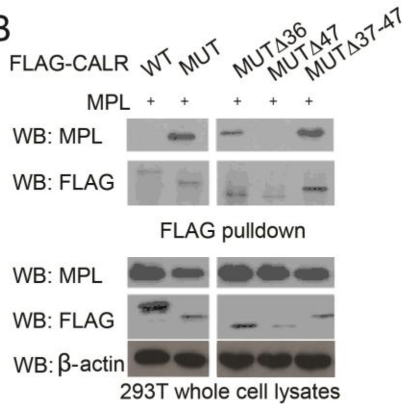
*CALR protein purification.* CALR variants were cloned into the pBAD-DEST49 vector (Invitrogen) and transformed into BL21 DE3 bacterial cells (Agilent). Protein expression was induced with 0.02% arabinose, and proteins were purified using the ProBond Purification System (Invitrogen).

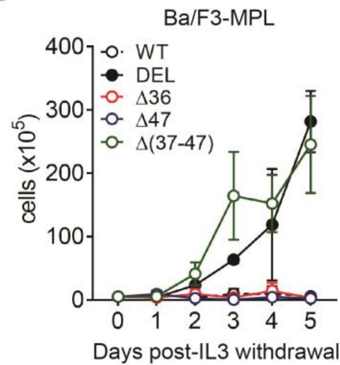
*In Vitro Binding Assay.* CHO-derived recombinant MPL was purchased R&D Systems. E. coli-derived recombinant ERp57 was purchased from Abcam.

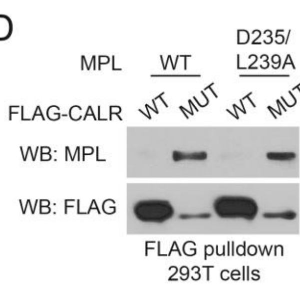
**Supplemental Figure 1**

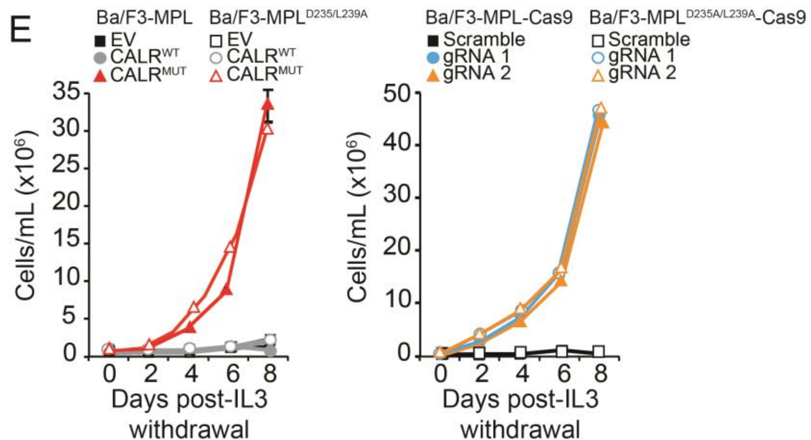
A

**52 bp del** QDEEQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA
**52 bp delΔ10** QDEEQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCR
**52 bp delΔ18** QDEEQRTRRMMRTKMRMRRMRRTRRKMRRKMSP
**52 bp delΔ28** QDEEQRTRRMMRTKMRMRRMRRT
**52 bp delΔ36** QDEEQRTRRMMRTKM
**52 bp delΔ47** QDEE
**52 bp delΔ37-47** QDEE _______________ RMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA




(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 Δ47 or Δ37-47 demonstrates that only deleting the 11 amino acid positively-charged stretch (QRTRRMMRTKM) 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 Δ47 or Δ37-47

demonstrates that only deleting the 11 amino acid positively-charged stretch (QRTRRMMRTKM) 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**

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