ARTICLE - Myeloproliferative Disorders

Loss of KDEL function from a calreticulin frameshift mutation drives expression of an immature, mutant calreticulin-dependent form of the thrombopoietin receptor MPL

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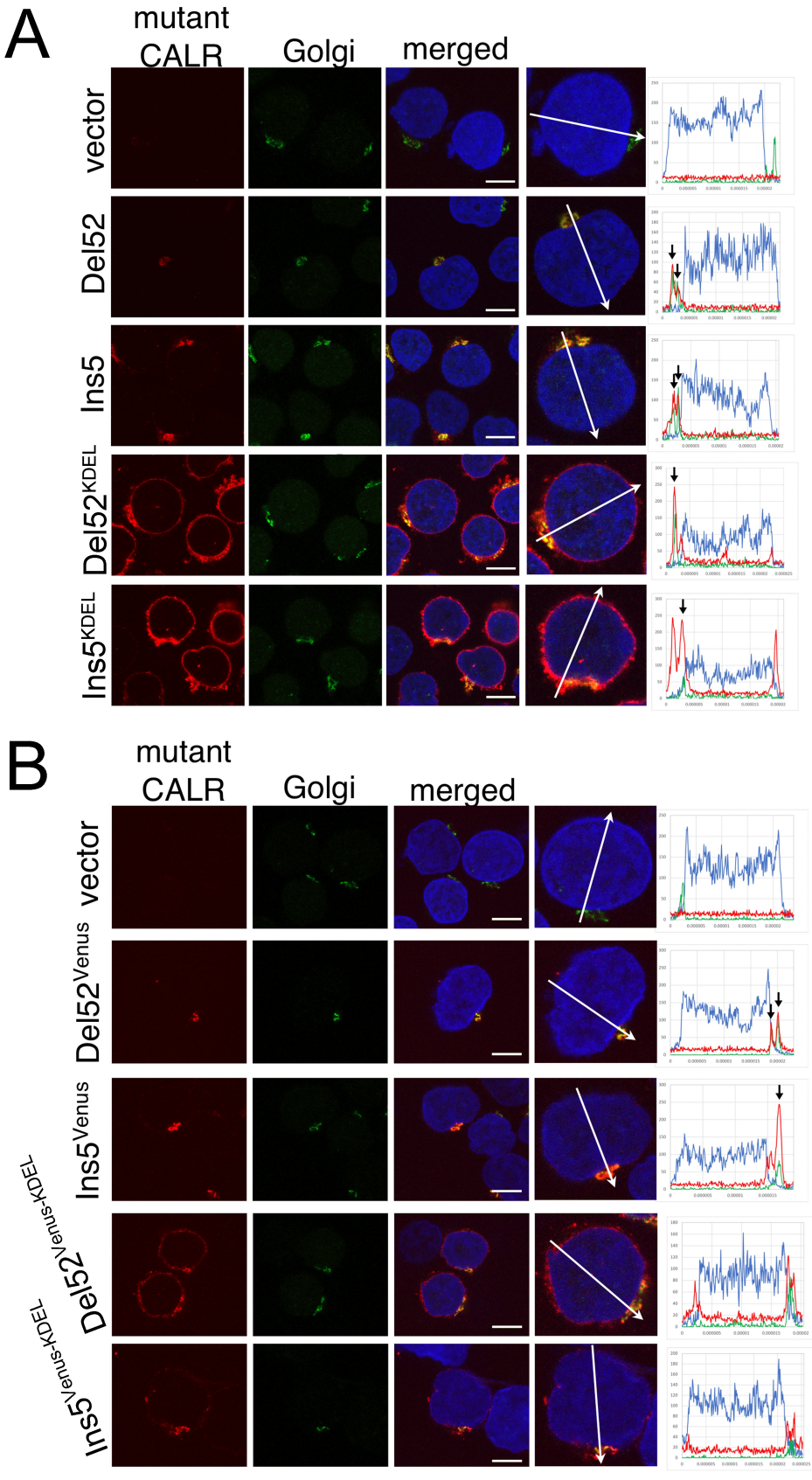
Supplementary Table 1: Primers used for the construction of CALR and MPL derivatives

Primer name	Primer sequence
CALR N-ter_Fw	5'-AAAAGAATTCGCCACCATGCTGCTATCCGTGCCGC-3'
CALRmut+KDEL_Rev	5'-TTTTCTCGAGCTACAGCTCGTCCTTGGCCTCAGTCCAGCCCTGG-3'
CALR N-ter_Fw	5'-AAAAGAATTCGCCACCATGCTGCTATCCGTGCCGCTGCTG-3'
CALRmut+linker Rev(Ron66)	5'-TGATCCTCCACCACCAGATCCACCTCCACC GGCCTCAGTCCAGCCCTGG-3'
Linker+Venus Fw (Ron49)	5'-GGTGGAGGTGGATCTGGTGGTGGAGGATCAATGGTGAGCAAGGGCGAGG-3'
Venus+KDEL Rev(Ron94)	5'-TTTTACCGGTCTACAGCTCGTCCTTCTTGTACAGCTCGTCC-3'
Venus Rev	5'-TTTTACCGGTTTACTTGTACAGCTCGTCCATGCCGAGAG-3'
CMV	5'-CGCAAATGGGCGGTAGGCGTG-3'
CALR WT$^{\Delta KDEL}$_Rev	5'-TTTCTCGAGCTAGGCCTGGCCGGGGACATCTTCC-3'
CMV	5'-CGCAAATGGGCGGTAGGCGTG-3'
CALR WT$^{\Delta KDEL}$+Flag_Rev	5'-TTTATCGTCATCGTCTTTGTAGTCGGCCTGGCCGGGGACATCTTC-3'
Flag+KDEL_Rev	5'-TTGAATTCCTACAGCTCGTCCTTTTTATCGTCATCGTCTTTGTAG-3'
CALR^{Y109F} _Fw	5'-CTGTGGGGGCGGCTTTGTGAAGCTGTTTC-3'
CALR^{Y109F} _Rev	5'-GAAACAGCTTCACAAAGCCGCCCCCACAG -3'
CALR^{D135L} _Fw	5'-CATGTTTGGTCCCCTCATCTGTGGCCCTG-3'
CALR^{D135L} _Rev	5'-CAGGGCCACAGATGAGGGGACCAAACATG-3'
MPL^{N117Q}_Fw	5'-GTGTTCCTACAGCAGACTCGGACTCAGCGAGTCC-3'
MPL^{N117Q}_Rev	5'-CGAGTCTGCTGTAGGAACACATTCTTCACCCAG-3'
MPL^{N178Q}_Fw	5'-GATCCCAAGCAGTCCACTGGTCCCACGGTCATACAG-3'
MPL^{N178Q}_Rev	5'-CCAGTGGACTGCTTGGGATCTCTGGGGCCATAGC-3'
MPL^{N298Q}_Fw	5'-GACCTGAAGCAGGTTACCTGTCAATGGCAGCAAC-3'
MPL^{N298Q}_Rev	5'-CAGGTAACCTGCTTCAGGTCCAAGGTAAAGCATTGC-3'
MPL^{N358Q}_Fw	5'-CAAGTCACGACAGGACAGCATTATTCACATCCTTG-3'
MPL^{N358Q}_Rev	5'-GCTGTCCTGTCGTGACTTGAAGTGGCAGCGAGAG-3'
CALR N-ter_Fw	5'-AAAAGAATTCGCCACCATGCTGCTATCCGTGCCGCTGCTG-3'
CALR mut+6-His_Rev	5'-AAGAATTCTCAGTGATGGTGATGGTGATGGGCCTCAGTCCAGCCCTGG -3'

Supplemental Figure 1 Masubuchi et al.



Supplemental Figure 2

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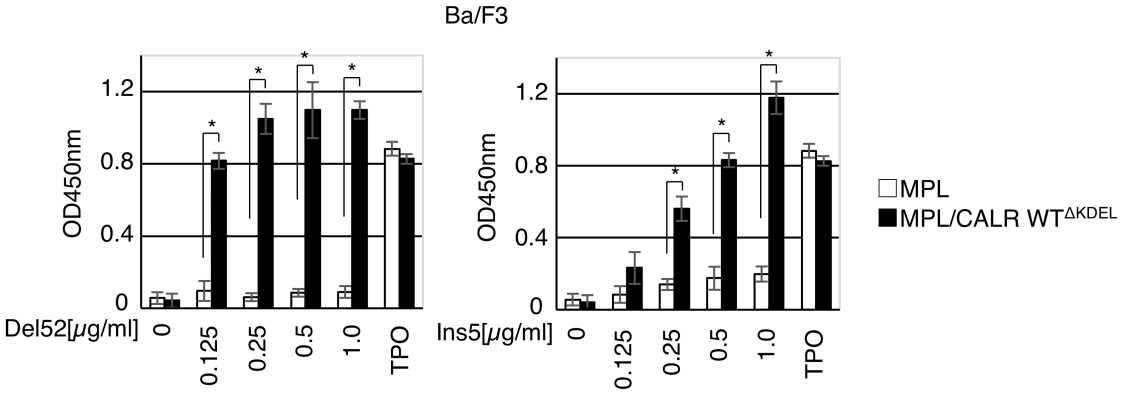

Supplementary Figure 1: (a) Confocal fluorescence images of UT-7/TPO vector, CALR Del52, CALR Ins5, CALR Del52^{KDEL}, and CALR Ins5^{KDEL} cells following immunofluorescence staining for mutant CALR, GM130 (Golgi apparatus), and nuclei. Scale bar: 10 μm. Intensity profiles of each fluorescent signal along the white arrows in the merged images are shown as line graphs using the corresponding colors. Black arrows indicate colocalization between mutant CALR and GM130 signals. (b) Confocal fluorescence images of UT-7/TPO vector, CALR Del52^{Venus}, CALR Ins5^{Venus}, CALR Del52$^{Venus\text{-}KDEL}$, and CALR Ins5$^{Venus\text{-}KDEL}$ cells after immunofluorescence staining for mutant CALR, GM130, and nuclei. Scale bar: 10 μm. Intensity profiles of each fluorescent signal along the white arrows in the merged images are shown as line graphs using the corresponding colors. Black arrows indicate colocalization between mutant CALR and GM130 signals.

Supplementary Figure 2: Cell proliferation assay in the absence or presence of recombinant mutant CALR or TPO in Ba/F3 MPL CALR WT$^{\Delta KDEL}$ cells after three days of culture. Absorbance was measured at 450 nm to detect the formazan dye produced by viable cells after 3 days. Data are presented as the mean ± SD of three replicates. The experiment was independently repeated three times with similar results, and representative data are shown. *p<0.05 was considered statistically significant.

Supplemental Information

Supplemental methods

Plasmids

To express untagged MPL, cDNA was subcloned into the pMSCV-IRES-DsRed FP vector (Addgene #52110).

Cell culture and proliferation assay

Interleukin (IL)-3-dependent Ba/F3 cells were cultured at 37°C in 5% CO_2 in Roswell Park Memorial Institute 1640 medium (Nacalai Tesque, #30264-56) containing 10% fetal bovine serum (Thermo Fisher, #10270-106), 1 ng/ml murine IL-3 (PEPROTECH, #213-13-50UG), 100 U/mL penicillin (Nacalai Tesque, #26239-42), and 100 μg/mL streptomycin (Nacalai Tesque, #32204-92). The cell proliferation assay and transfection were performed as previously described[3].