iScience, Volume *24*

Supplemental information

Reduced eIF4E function impairs B-cell leukemia without altering normal B-lymphocyte function

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Supplementary Figures:

Supplementary Figure 1. **Genetic deletion of eIF4E reduces cap-dependent translation in p190 cells, Related to Figure 1**

A. Renilla and Firefly luciferase cap-dependent translation readings of p190s from WT or Het mice treated with vehicle or MLN100nM.

B. Table of the Renilla and Firefly counts.

C. Puromycin incorporation assay of eIF4E+/+, eIFE4 fl/+ and eIF4E fl/fl p190 treated with vehicle, cycloheximide (CHX) or 4OHT (1μM). % incorporation was quantified using Flowjo software (10.7.1). Data are expressed as mean +/- SEM. (* $p < 0.05$, ** $p < 0.01$, ***$p < 0.001$, one way ANOVA, n = 2-4 per group).

Supplementary Figure 2. **Deletion of eIF4E reduces p190 growth, Related to Figure 2**

A. Colony formation count of WT and $eIF4E^{+/-}$ p190. Data are expressed as mean +/- SEM. (** $p < 0.01$, unpaired t-test, n = 4)

B. Representative flow cytometry plots of hCD4 and GFP WT and Het cells mixed at equal ratios early after infection with p190 BCR-ABL when ~30% of the mix was positive for each marker before in vivo injection.

C. Competitive growth assay of established hCD4 and GFP p190 $eIF4E^{fl/fl}$ Cre+ treated with 1μM 4OHT. Data are expressed as number of hCD4 and GFP marker positive cells measured by flow cytometry.

D. Protein analyses of eIF4E, 4EBP-1 and t-ERK in hCD4 and GFP $eIF4E^{+/+}$, $eIF4E^{fl/+}$ p190 after 72h of 4OHT treatment.

Supplementary Figure 3. Resting mouse splenic B cells are not affected by genetic deletion of eIF4E, Related to Figure 3.

A. Gating strategy for splenic B subset

B. quantification of two mature (FO and MZ) and three transitional subsets (T1, T2 and T3) in WT and Het mice measured by flow cytometry. Data are expressed as percentage and total cell number +/- SEM. n = 3.

C. Western blot of protein expression in B cells from a representative pair of WT and $eIF4E^{+/-}$ mice. In 4 out of 6 pairs analyzed, eIF4E expression was approximately 50% reduced in the $eIF4E^{+/-}$ cells. In two other pairs, eIF4E expression was less reduced for unknown reasons.

D. RT-qPCR measurements of eIF4E, 4EBP-1 and 4EBP-2 mRNA transcripts in purified WT or $eIF4E^{+/-}$ B cells with or without LPS+IL-4 stimulation. Data are expressed as mean +/- SEM. of fold change using WT vehicle treated. Significance was calculated using unpaired one-tailed student's t-test (***$p < 0.001$, n = 3).

E. Renilla and Firefly luciferase cap-dependent translation readings of WT and $eIF4E^{+/-}$ B cells stimulated with LPS+IL-4 for 48hours.

Supplementary Figure 4. Progenitor B cell survival is not affected by genetic deletion of eIF4E, Related to Figure 3 and 4.

A. Representative plots of progenitor B cell subsets ($B220^{+}CD43^{+}$ and $B220^{+}CD43^{-}$) of WT and $eIF4E^{+/-}$ BM cells cultured *ex vivo* in presence of IL-7. Live cells were gated as $7AAD^{-}$.

B. Percentage of viable pro-B cells ($B220^{+}CD43^{+}7AAD^{-}$) +/- IL-7 or treated with different concentrations of Rapamycin (Rapa 0.2-2-20nM) or MLN0128 (MLN 1-10-100nM). Data are expressed as mean +/- SEM. (*$p < 0.05$, ** $p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, one way

ANOVA, n = 3)

C. Representative plot of progenitor B cell subsets ($B220^+CD43^+$ and $B220^+CD43^-$) of eIF4E fl/+ and +/+ BM cells cultured *ex vivo* with or without of IL-7. Live cells were gated as $7AAD^-$.

D. Percentage of viable $B220^+CD43^+7AAD^-$ of fl/+ and +/+ BM cells treated without IL-7, +IL7 -4OHT, +IL7 + 4OHT (1μM) and +IL7 +MLN0128 100nM. Data are expressed as percentage of live cells positive for the $B220^+CD43^-$ phenotype. N = 3.

Supplementary Figure 5. *In vivo* and *in vitro* antibody production is not affected by genetic deletion of eIF4E, Related to Figure 3.

A.-B. Antibody measurements of WT and $eIF4E^{+/-}$ mice immunized with sheep red blood cells (SRBC). N = 5 mice per group. Measurement of SRBC-specific IgG1 and IgM was done by flow cytometry as described (McAllister *et al.*, 2017).

A. Measurements of SRBC-specific IgG1.

B. Measurements of SRBC-specific IgM. Data are expressed as mean fluorescence intensity (MFI) +/- SEM. (*$p < 0.05$, ** $p < 0.01$, ****$p < 0.0001$, two-way ANOVA)

C. WT and $eIF4E^{+/-}$ isolated splenic B cells were stimulated with LPS+IL-4 and percent of live B cells that have divided at least once expressing IgG1 were measured at 72 hours by flow cytometry.

D. Percentage of dividing IgG1 expressing WT and $eIF4E^{+/-}$ B cells stimulated with αCD40+IL-4, 5 μg/mL LPS+IL-4,or 0.5 μg/mL LPS+IL-4 and treated with indicated concentrations of rapamycin was measured at 72 hours by flow cytometry.

Supplementary Figure 6. $eIF4E^{fl/+}$ splenic B cells have normal proliferative capacity,

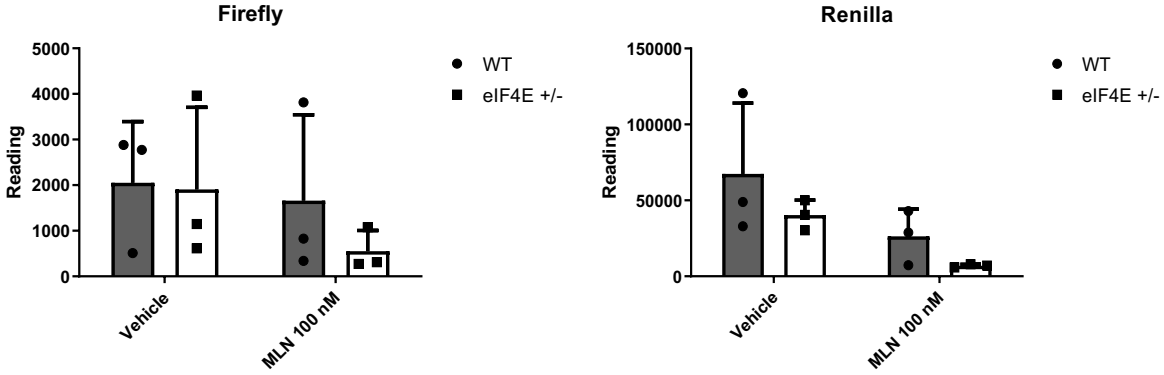
Related to Figure 4.

A. Experimental scheme of competitive proliferation assay of WT or eIF4E$^{fl/+}$ B cells stained with CFSE or CellTrace FR.

B. Gating strategy for purified eIF4E$^{+/+}$, eIF4E$^{fl/+}$ B cells labeled with CFSE or CellTrace FR (630nM) +/- 4OHT and +/- 10μg/ml anti-IgM and 10ng/ml IL-4. For each FACS plot, the red and orange histograms represent the unstimulated cells pretreated without (red) or with (orange) 4OHT. The blue and green histograms are the stimulated cells pretreated without (blue) or with (green) 4OHT. Note that the blue and green histograms are essentially overlapping in all eight FACS plots, indicating that deletion of one allele of *Eif4e* does not impact B-cell division potential. The vertical boxes represent the cell events used to calculate % dividing cells in Figure 4C. The peaks with low fluorescence to the left of these boxes are background fluorescence of cells in the mixture that were not labeled with the dye being measured. Background fluorescence is higher in the blue and green traces, presumably due to increased cell size upon mitogen stimulation.

Supplementary Figure 1

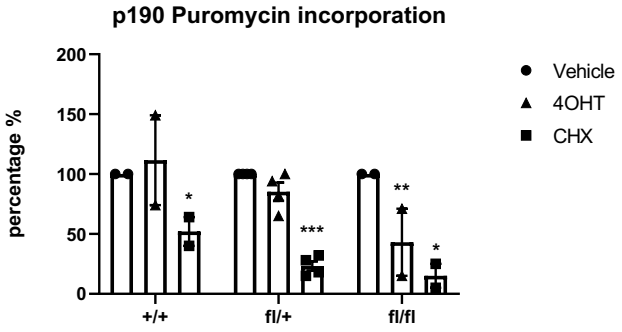
(A)



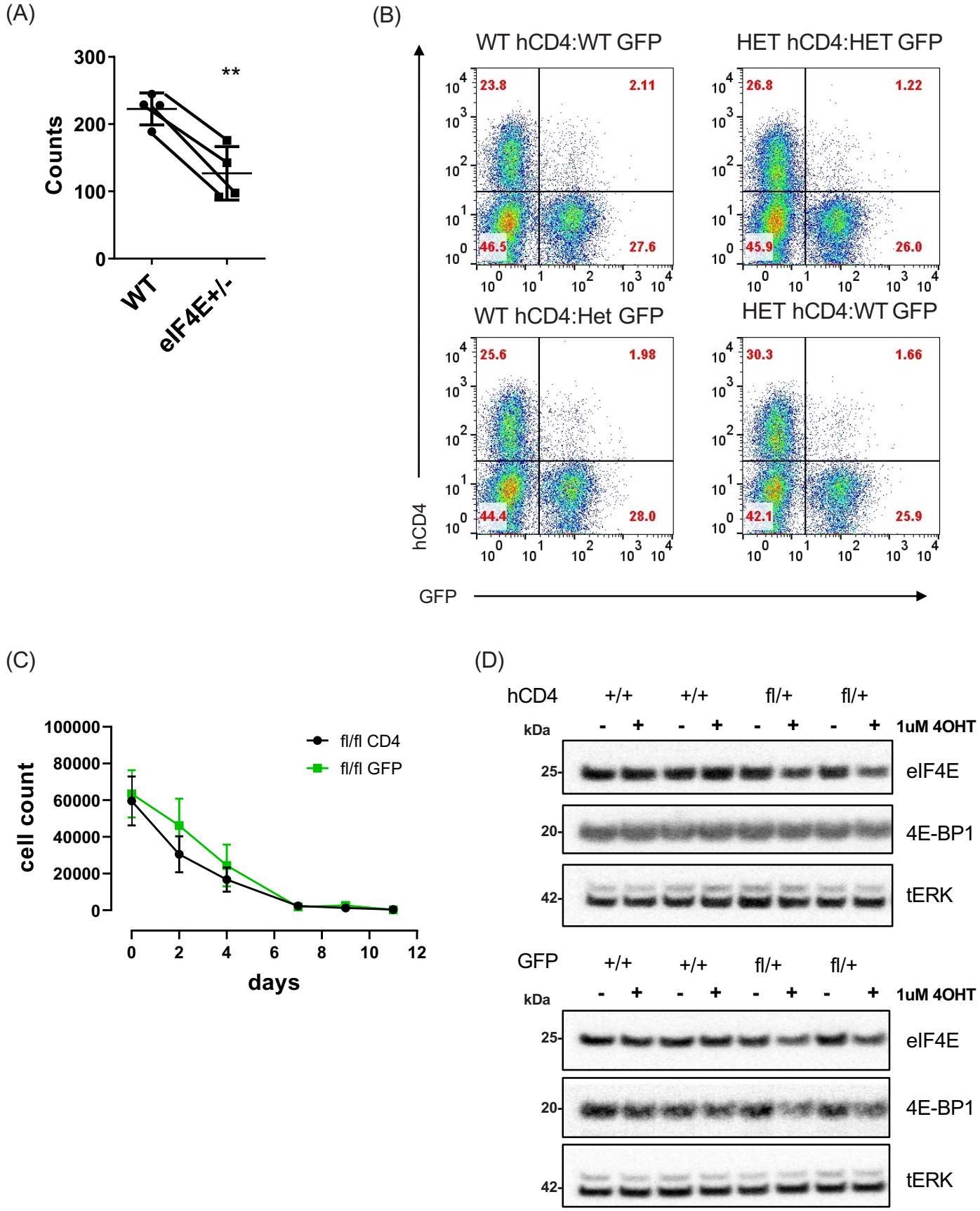
(B)

		Vehicle			MLN 100nM		
Firefly	**WT**	2881	509	2772	3816	339	827
	eIF4E +/-	3963	614	1148	1076	310	272
Renilla	**WT**	48962	32983	120528	42894	7339	28781
	eIF4E +/-	50044	30352	40484	5844	7884	7006

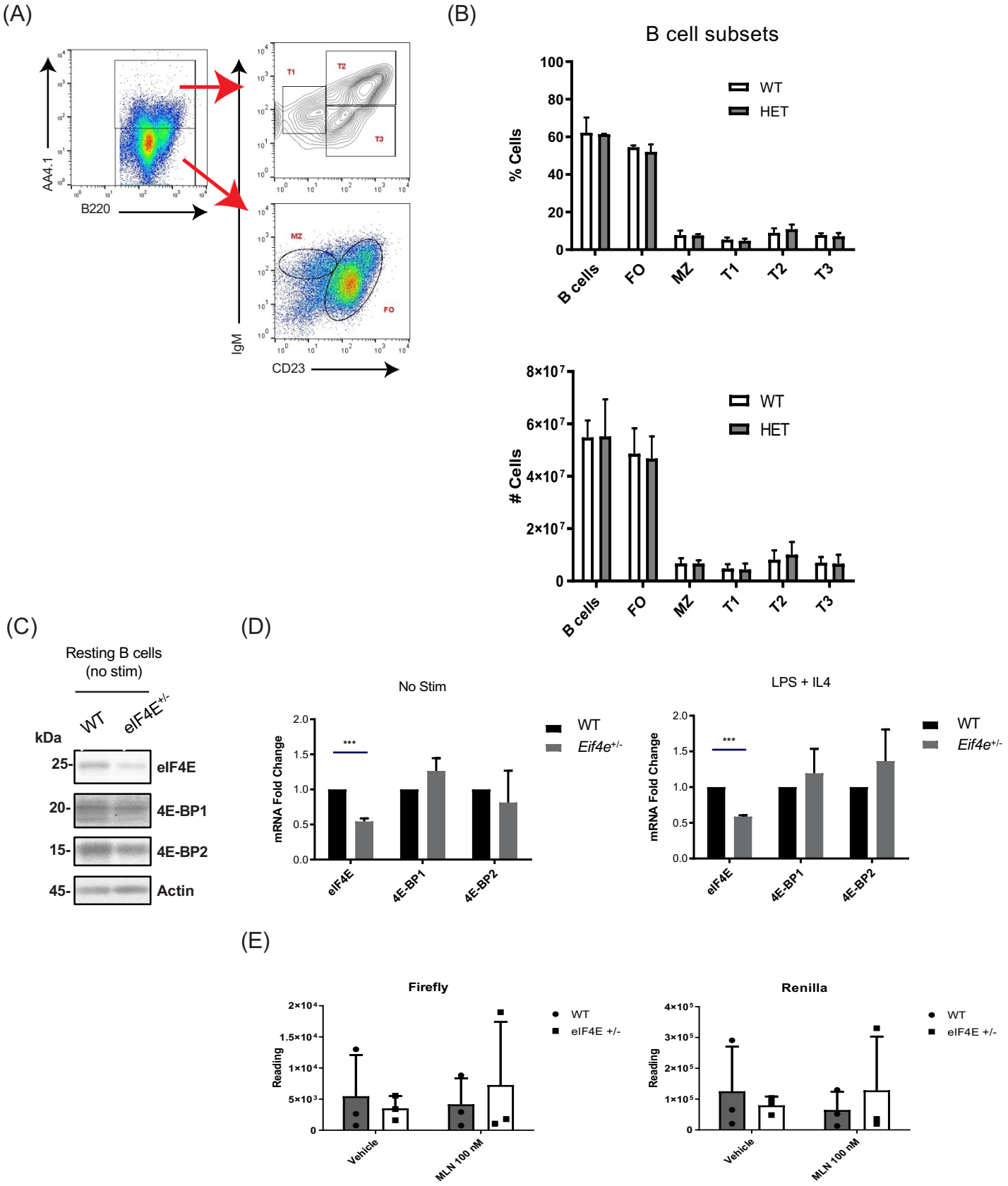
(C)



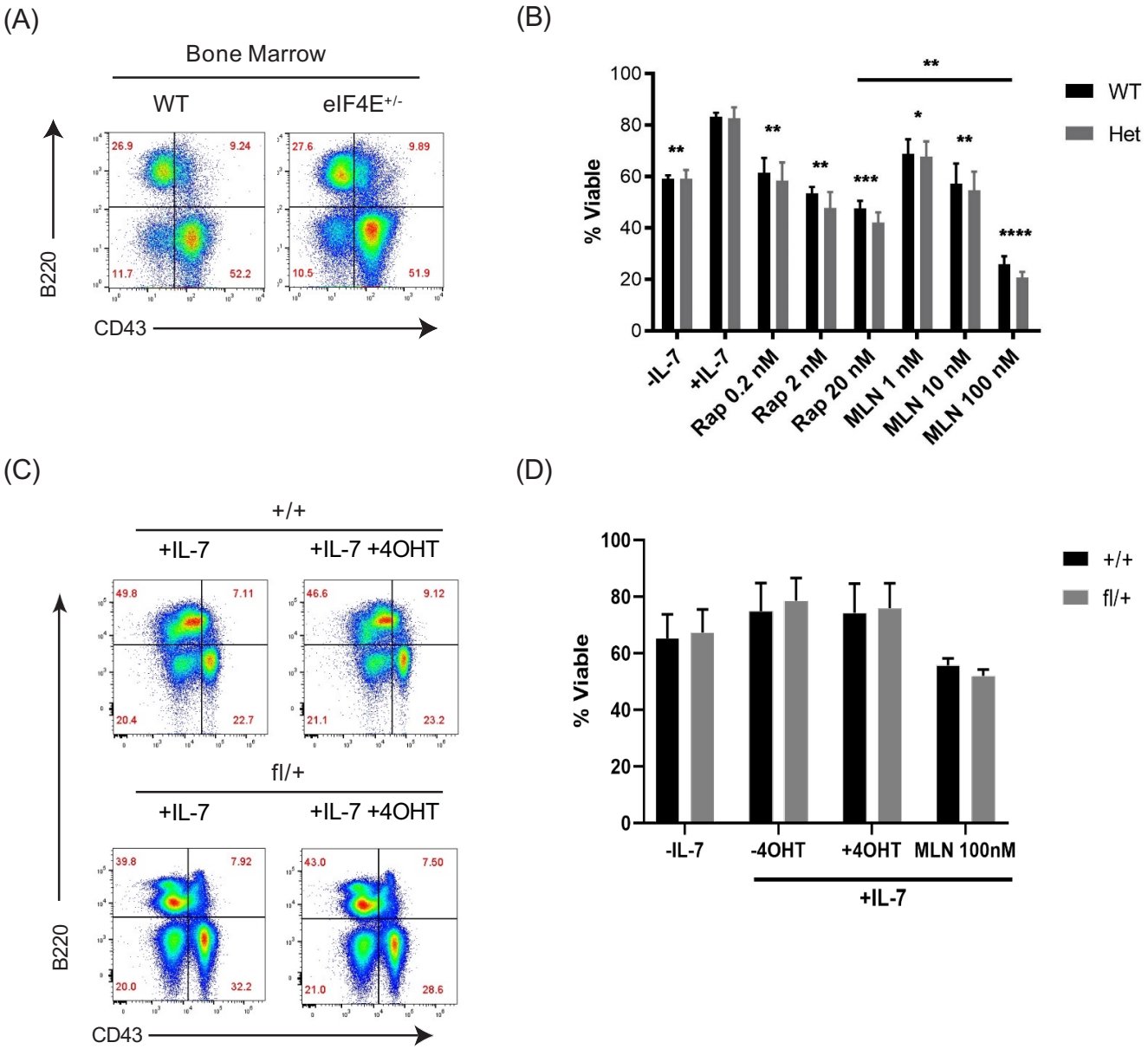
Supplementary Figure 2



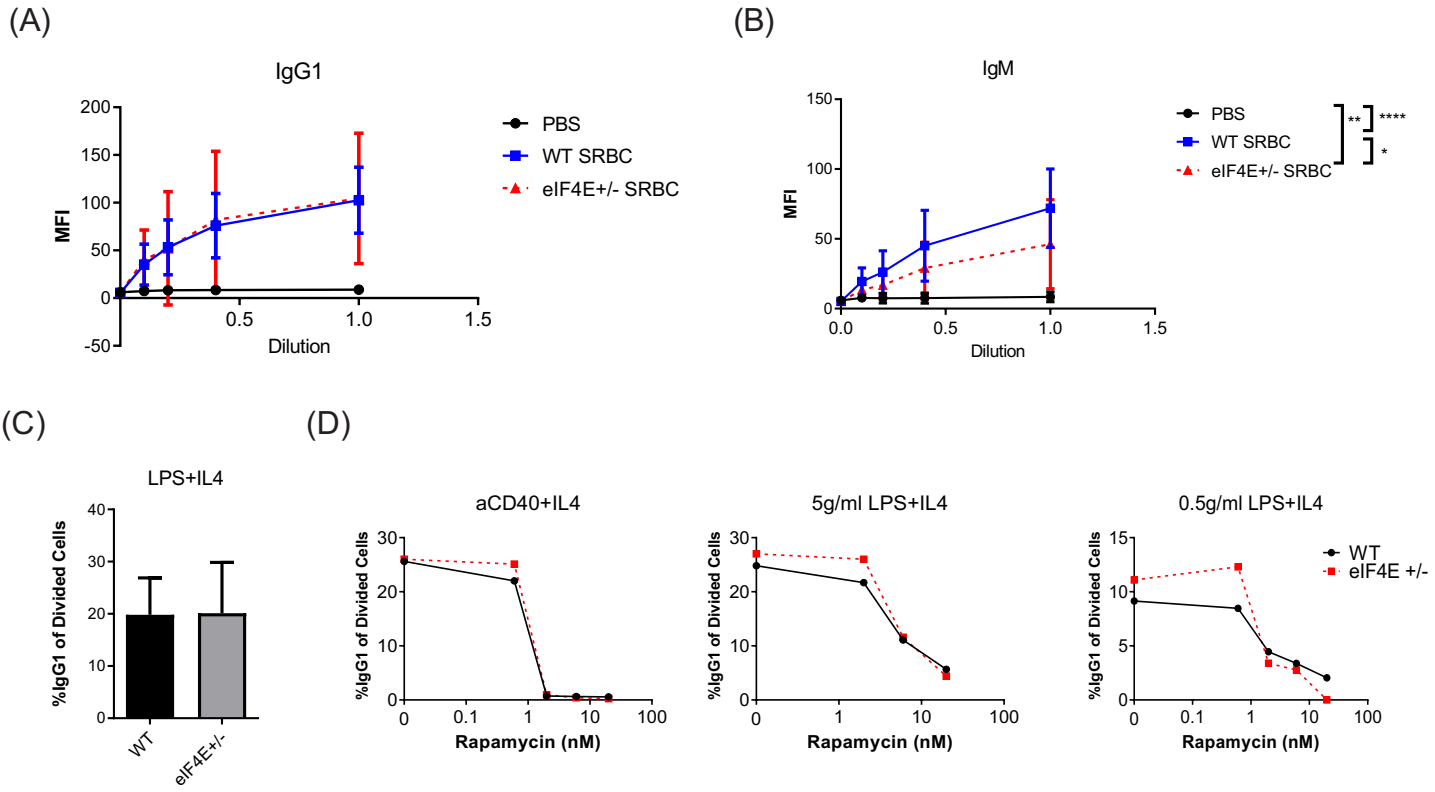
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6

(A)

(B)

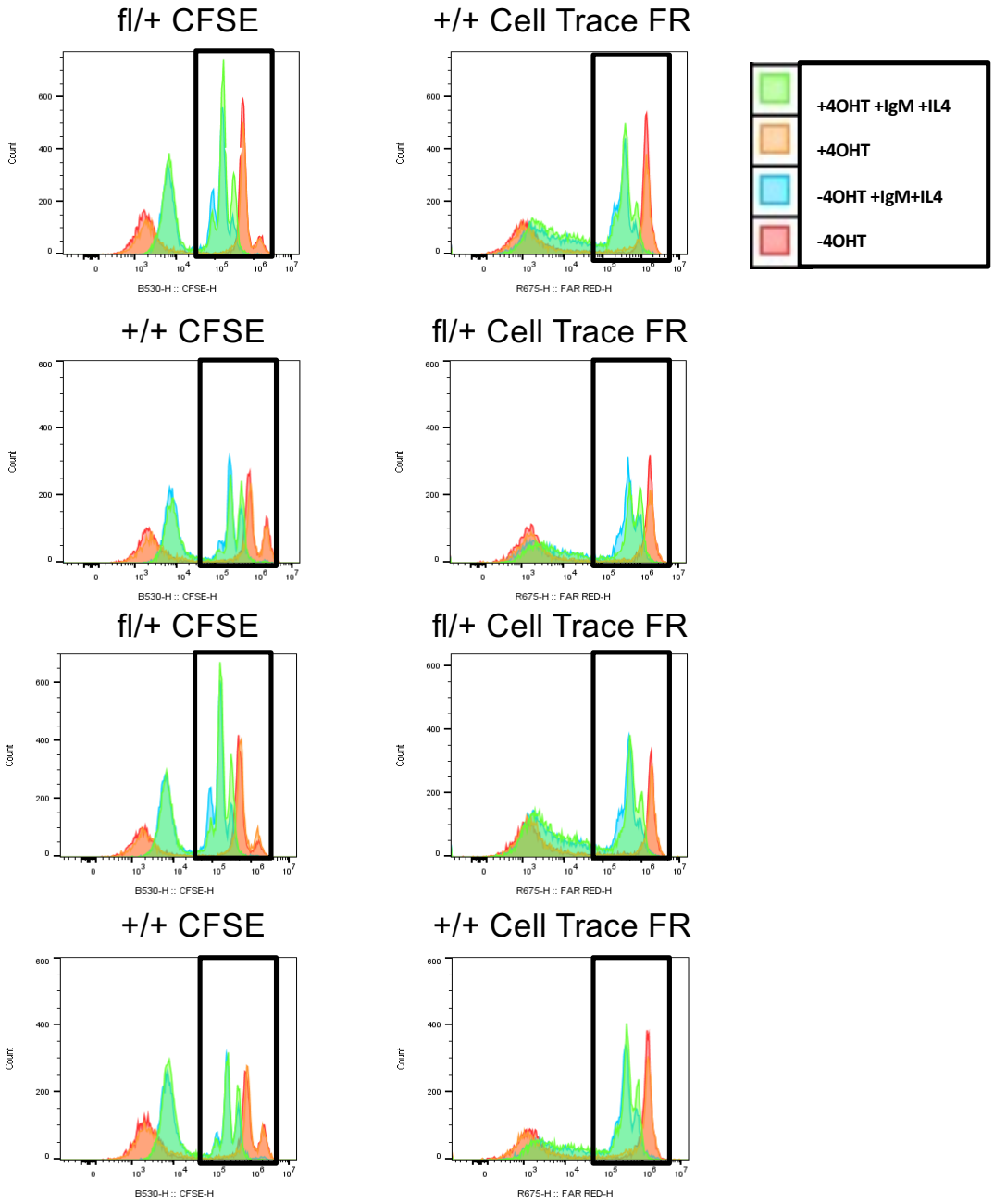


Table S1: Oligonucleotide sequences of primers used for genotyping *Eif4e*-flox and Cre strains

Primers	Forward	Reverse
eIF4E set1	AGAGCAAATACGGAACCGACGTGTGC	ATGCAGGGTTTGGGTGCTTACACAG
eIF4E set2	GAGCAAATACGGAACCGACGTGTGC	GAAGTTATCTCGACGAAGTTCC
eIF4E set3	GCGCAACGCAATTAATGATAAC	TCTGCTAGCTTGTTCTCACGCACCC
Cre	GCGGTCTGGCAGTAAAAACTATC	GTGAAACAGCATTGCTGTCACTT