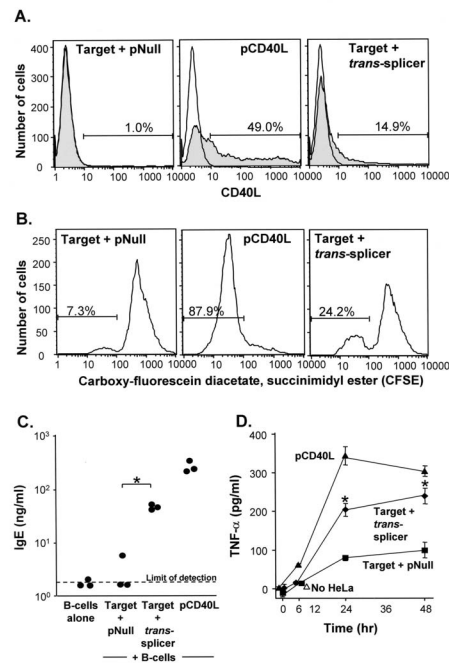


Supplemental  
Figure 3



**Supplementary Figure 3.** Assessment of restoration of function following *in vitro trans*-splicing-mediated correction of CD40L pre-mRNA. **a.** Flow cytometric analysis of *trans*-spliced CD40L in HeLa cells. Analysis was performed 36 hr after co-transfection with the target plasmid plus either *trans*-splicer or null (pNull) plasmids. Transfection with an intact plasmid expressing the intact CD40L cDNA was used as positive control. Normalized histograms are shown for isotype-matched negative control (solid line) and anti-CD40L antibodies (gray area). The number of HeLa cells positive for CD40L per  $10^4$  cells analyzed is shown on the ordinate. **b.** Stimulation of murine B cell division by *trans*-spliced CD40L in HeLa cells. Thirty-six hr after co-transfection as described in panel **a.**, HeLa cells were irradiated, B cells were harvested from CD40L wild type spleens and stained with carboxy-fluorescein diacetate, succinimidyl ester (CFSE). B cells ( $10^5$  cells/well) were co-cultured with irradiated HeLa ( $10^5$  cells/well), cultured with IL-4 in flat bottom 96 well plates, and analyzed 7 days after by flow cytometry for B220-positive cells. Percent of B cell proliferation is shown on each panel. **c.** IgE production from B cells stimulated by CD40L *trans*-splicing in HeLa cells. Co-transfected and irradiated HeLa cells ( $10^5$  cells/well) were mixed with B cells from a CD40L wild type mouse ( $10^5$  cells/well) and co-cultured in the presence of IL-4 and IL-5 in flat bottom 96 well plates. After six days, the concentration of IgE in the conditioned medium was measured by ELISA. Each circle shows one analysis. \*,  $p < 0.005$ , target + *trans*-splicer vs target + pNull. **d.** TNF- $\alpha$  production from RAW 264.7 macrophage stimulated by CD40L *trans*-splicing in HeLa cells. Time course of TNF- $\alpha$  secretion from RAW 264.7 cells co-cultured with irradiated HeLa cells as described in panel B with target + *trans*-splicer (v), pCD40L ( $\Delta$ ), target + pNull (v) or no HeLa cells ( $\square$ ). Data represent mean  $\pm$  standard error ( $n = 3$  per data point). \*,  $p < 0.01$ , target + *trans*-splicer vs target + pNull.