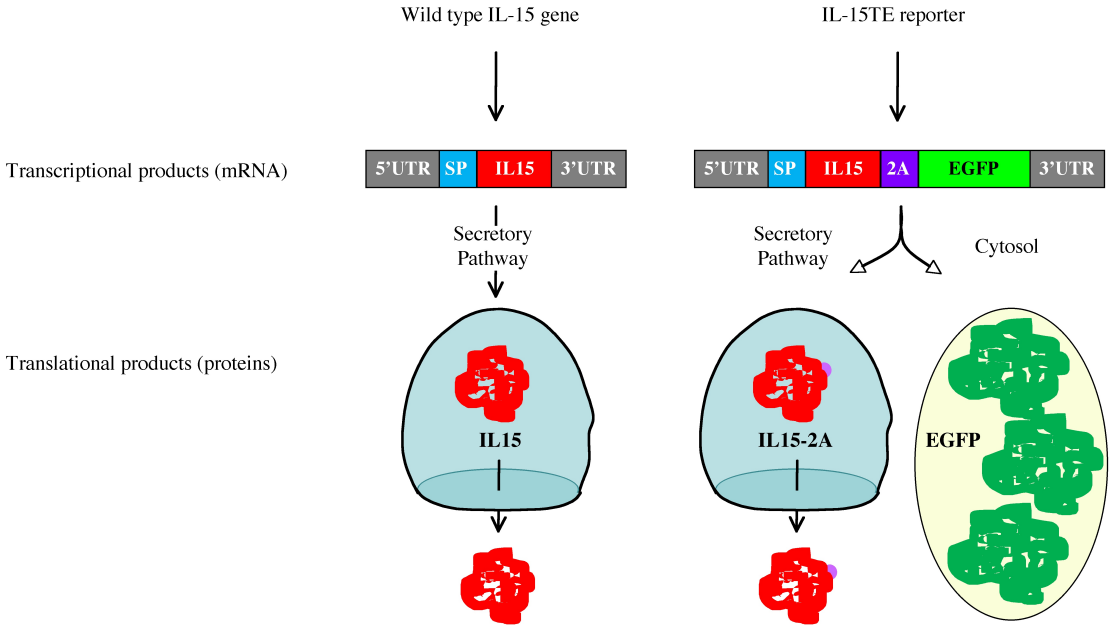
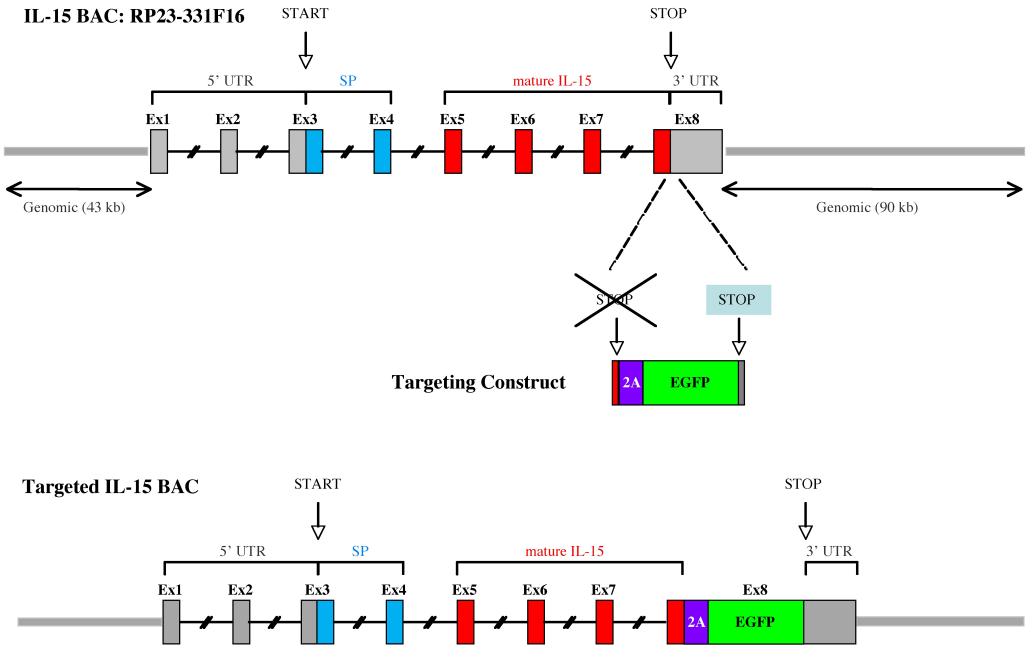
Supplemental Figure 1a



Supplemental Figure 1b



Supplemental Figure 1. A) Rationale for construction of translational reporter for IL-15. Studies from Waldman group implicated several regulators of translation contained within the 5'untranslated region (UTR), in the signal peptide (SP), and in the coding region of IL-15 . In order to generate a reporter that truly reflects all aspects of IL-15 regulation of expression, we included all these elements and also added native 3'UTR. To preserve translational control, we utilized the functionality of a viral 2A-peptide that allows for co-translational expression of two polypetides from a single mRNA . We incorporated a modified sequence of a 2A peptide from Thosea asigna virus, called TaV(CtoS), between the end of IL-15 coding sequence and Enhanced Green Fluorescence Protein (EGFP) (see Materials and Methods). This reporter construct encodes a single open-reading-frame of IL-15/2A-TaV/EGFP(IL-15TE). 2A peptide causes translational pause that results in hydrolysis of the nascent IL-15 polypeptide, followed by translation of EGFP. This tight coupling of translation allows for monitoring of IL-15 production by detection of EGFP. In addition, IL-15 protein is translated into the secretory pathway since it has a signal peptide, while EGFP accumulates in the cytosol. This phenomenon has been reported by de Felipe and confirmed by us (data not shown). Retention of the reporter protein in the cytosol may increase sensitivity of detection. B) Generation of IL-15 translational reporter mouse IL-15TE. Given that production of IL-15 protein is controlled transcriptionally and translationally , we generated a transgenic reporter mouse that would reflect both levels of this regulation. To preserve transcriptional control, we selected a Bacterial Artificial Chromosome (BAC) RP23-331F16 that contained the intact genomic locus of IL-15 flanked by large regions of genomic DNA (43 kb upstream and 90 kb downstream, respectively), as the backbone for our transgenic construct. To preserve translational control, we utilized the functionality of a viral 2A-peptide (see above). Thus, our targeting construct contained in-frame sequences of IL-15, 2A, and EGFP, followed by a short stretch of IL-15 3'UTR. The resulting targeted BAC encoded a single open-reading-frame of IL15/2A/EGFP.