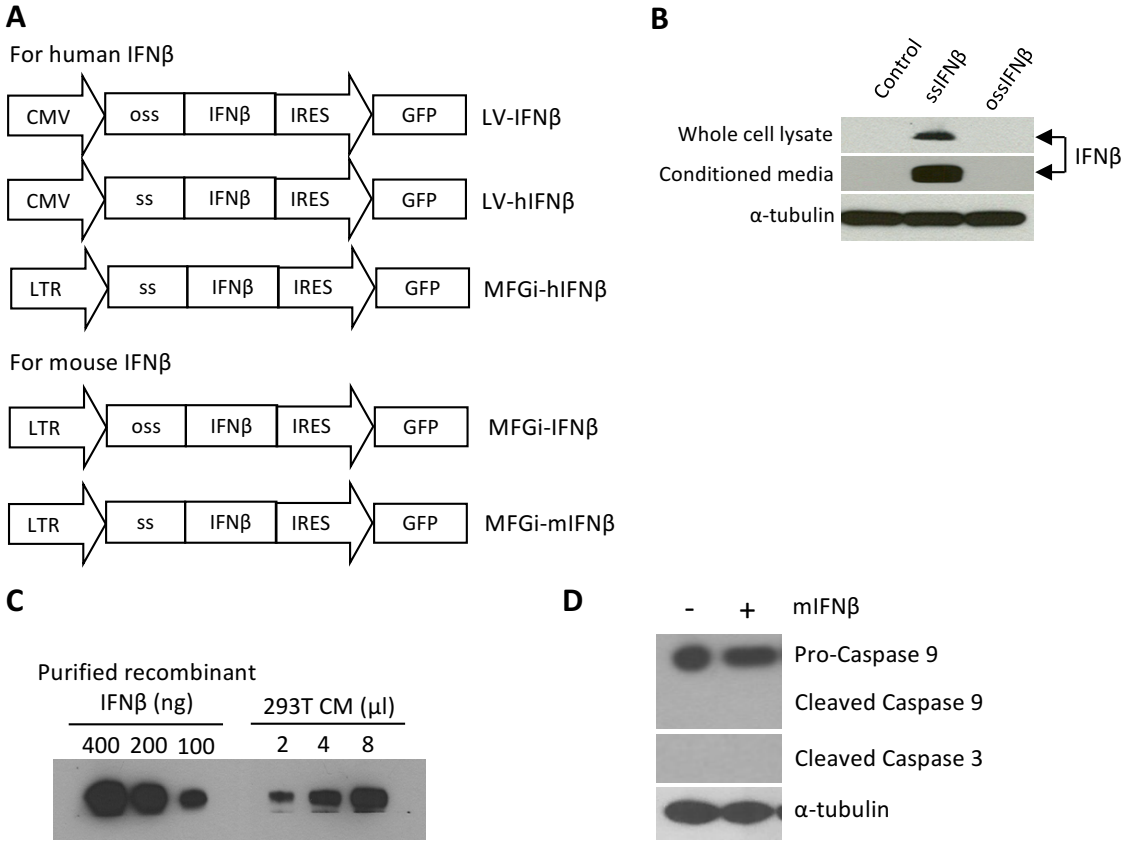

Supplemental Figure 7. Engineering cells expressing secretory mouse and human IFNβ.
(A) Design of retroviral vector expressing secretable human and murine IFNβ. The complementary DNA of hIFNβ and mIFNβ was cloned after the CMV promoter in CSCGW lentiviral vector or after the LTR promoter in MFGi-GFP, which also bears an internal ribosomal entry site (IRES) and GFP. The signal sequence of IFNβ (oss) was replaced with that of Flt3 ligand (ss) to maximize secretion of mIFNβ. (B) HEK-293T cells were transfected by LV-GFP (control), lentiviral vectors expressing ssIFNβ (with Flt3L signal sequence) or ossIFNβ (with it endogenous signal sequence). Western blot analysis was performed to detect IFNβ from whole cell lysates and their conditioned medium. Conditioned medium was concentrated to equivalent volumes for each cell lysate. (C) To quantify the amount of IFNβ in conditioned medium, indicated amounts of conditioned medium from HEK-293T transfected with MFGi-hIFNβ (293T CM) were Western blotted and compared to purified recombinant human IFNβ, blotted in parallel. The concentration of hIFNβ in the conditioned medium was estimated by comparing hIFNβ band intensity to that of purified recombinant IFNβ. (D) Activation of Caspase 3 and Caspase 9 were checked by Western blot analysis on CT2A cell lysates collected 24 hours after treatment with mIFNβ.