Title: A polymorphic distal enhancer controls expression of interferon beta in murine and human myeloid cells

Background: Interferon β (IFN-β) is a central cytokine in the antiviral response that regulates the immune reaction to infections and tumors. We have previously shown that knocking out the gene coding for the TRIM33 chromatin reader in myeloid cells leads to uncontrolled expression of *Ifnb1* at the end of cellular activation.
Using TRIM33 ChIP-seq, and then 3c-seq based on the TRIM33 peaks, we have identified new genome loci that could play a role as myeloid-specific *Ifnb1* enhancers. These loci include a predicted super-enhancer in mice, and are conserved in humans. We report here the functional characterization of these putative enhancers in a luciferase reporter system, using NanoLuc (Nluc) as a normalization protein.

Results: We cloned the putative enhancers in front of the *Ifnb1* promoter into the pGL4.12 firefly luciferase (Fluc) vector. We first sought to transfect these constructs together with the pRL-TK normalization vector, coding for renilla luciferase (Rluc) under the control of the thymidine kinase promoter. These experiments were performed in a murine myeloid cell line, RAW264.7, that is hard to transfect. The levels of Rluc were not sufficient to allow reliable normalization. In addition, vectors coding for Rluc under the control of stronger promoters were not usable, as these promoters respond to lipopolysaccharide (LPS), a classical myeloid activator. We therefore tested the pNL1.1.TK vector, coding for Nanoluc (Nluc) under the control of the thymidine kinase promoter, together with the Nano-Glo® Dual-Luciferase® Reporter Assay System. The Nanoluc levels obtained after transfection were amply sufficient for normalization, allowing to increase the Fluc/Nluc plasmid ratio, hence the signal. In addition, the TK promoter did not respond to LPS, allowing to characterize the enhancers in conditions of cellular activation.

Conclusions: Four out of the six putative enhancers were constitutively active in murine myeloid cells, and three of them conferred a significantly better induction than the promoter alone after LPS activation. Our results show that the Nluc system allows to obtain reliable results in difficult cellular models.