resistant macrophages, to correct immune dysfunction linked to NOD2-deficient CD pathogenesis. We used in vitro and in vivo models of NOD2 deficiency to demonstrate the mechanism of action and the efficacy of OTL-104 autologous HSC-GT. NOD2-deficient human myeloid cells differentiated in vitro from CRISPR-generated NOD2-deficient CD34+ HSCs are unable to mount a proinflammatory cytokine response to MDP stimulation. Similarly, myeloid cells differentiated from CD34+ cells obtained from peripheral blood of genetically characterized NOD2-deficient CD patients, are also refractory to MDP stimulation and unable to generate a normal cytokine response profile (IL-8, TNFα, IL-6, CXCL1/2 and IL-10). In both NOD2 deficient CD34+ derived monocyte models, transduction with a lentiviral vector (LVV) expressing NOD2 fully restores NOD2-dependent cytokine and chemokine responses, restoring an immune profile that is comparable to monocytes derived from CD34+ cells from NOD2 wild-type healthy donors.

Transplantation of lineage negative (Lin) hematopoietic stem/progenitor cells (HSPCs) transduced with the OTL-104 LVV in Nod2−/− mice was used as an in vivo model of gene therapy for CD. Compared to WT mice, Nod2−/− mice fail to release systemic inflammatory mediators and recruit myeloid cells in response to MDP administration. Transplantation of transduced Lin HSPCs restores MDP-induced systemic release of IL-6 and CXCL1 as well as innate mobilization of monocyte/macrophage cells and gut associated immune markers. Transplanted Nod2−/− mice display normal hematopoiesis and stable vector copy numbers in hematopoietic cells.

Key to our therapeutic approach, histopathological analysis of intestinal lamina propria from transplanted mice shows a normal biodistribution and physiological NOD2 gene expression in tissue resident cells (Figure 1). These results confirm the impact of NOD2 deficiency in primary immune activation and demonstrates the therapeutic potential of OTL-104 HSC-GT for long-term correction of NOD2-deficient CD.

**Figure 1. HSC gene therapy restores a normal intestinal biodistribution of NOD2 gene expression.** In situ RNAscope NOD2 mRNA detection in intestinal lamina propria of WT and Gene Therapy (GT) treated NOD2−/− mice, confirming NOD2 gene expression is restored within hematopoietic derived tissue resident cells.