Hectd3 promotes pathogenic Th17 lineage through Stat3 activation and Malt1 signaling in neuroinflammation

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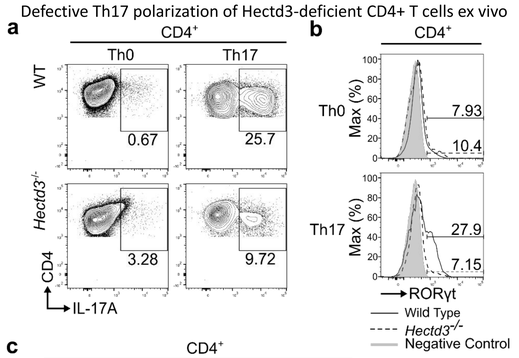
ABSTRACT

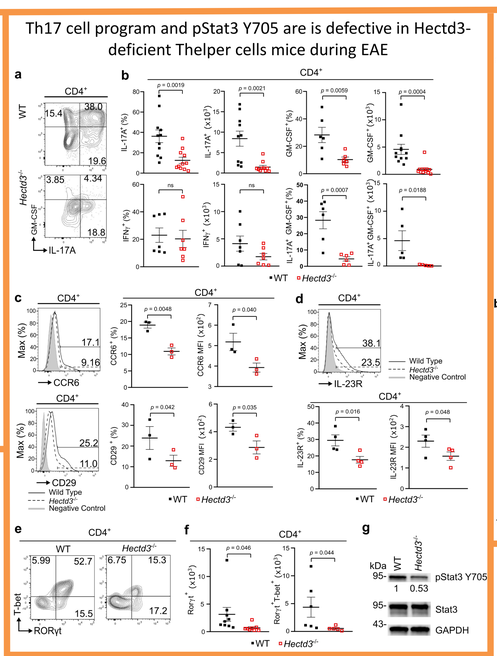
Polyubiquitination promotes proteasomal degradation, or signaling and localization, of targeted proteins. Here we show that the E3 ubiquitin ligase Hectd3 is necessary for pathogenic Th17 cell generation in experimental autoimmune encephalomyelitis (EAE), a mouse model for human multiple sclerosis. Hectd3-deficient mice have lower EAE severity, reduced Th17 program and inefficient Th17 cell differentiation. However, Stat3, but not RORγt, has decreased polyubiquitination, as well as diminished tyrosine-705 activating phosphorylation. Additionally, non-degradative polyubiquitination of Malt1, critical for NF-κB activation and Th17 cell function, is reduced. Mechanistically, Hectd3 promotes K27- and K29-linked polyubiquitin chains on Malt1, and K27-linked polyubiquitin chains on Stat3. Moreover, Stat3 K180 and Malt1 K648 are targeted by Hectd3 for non-degradative polyubiquitination to mediate robust generation of RORγt^{+}IL-17A^{hi} effector CD4^{+} T cells. Thus, our studies delineate a mechanism connecting signaling related polyubiquitination of Malt1 and Stat3, leading to NF-kB activation and RORγt expression, to pathogenic Th17 cell function in EAE.

RESULTS

Defective Th17 polarization of Hectd3-deficient CD4+ T cells ex vivo



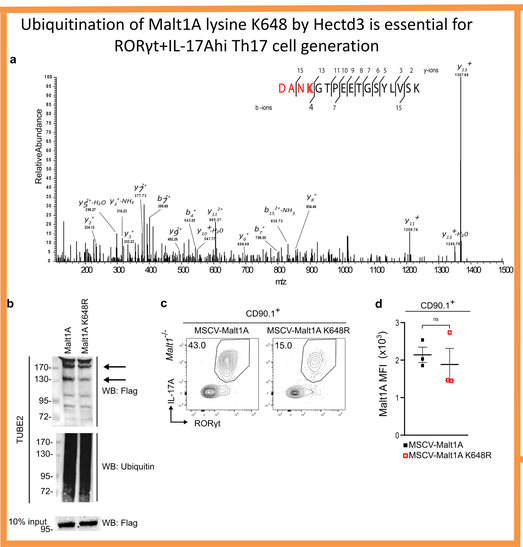
Th17 cell program and pStat3 Y705 are is defective in Hectd3-deficient Thelper cells mice during EAE



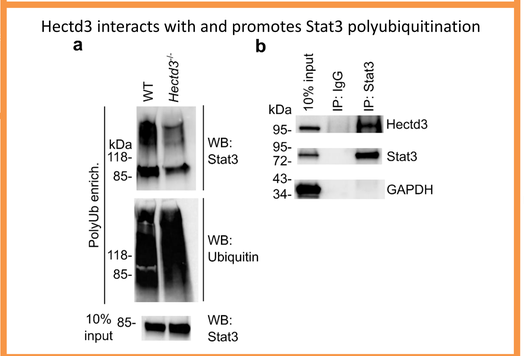
Hectd3-deficient Th17 cells have reduced pathogenicity

Hectd3-deficient mice have attenuated EAE severity with reduced CD4+ T cell infiltration in the CNS

Ubiquitination of Malt1A lysine K648 by Hectd3 is essential for RORγt+IL-17Ahi Th17 cell generation



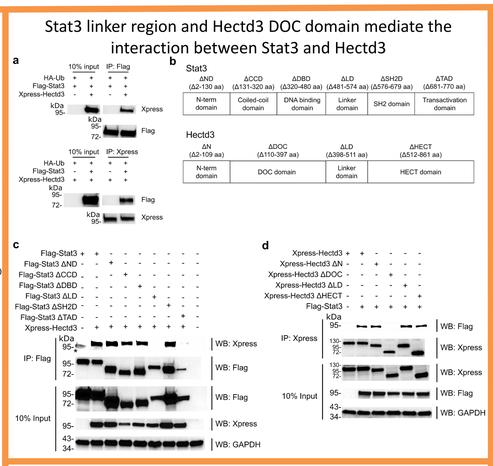
Hectd3 interacts with and promotes Stat3 polyubiquitination



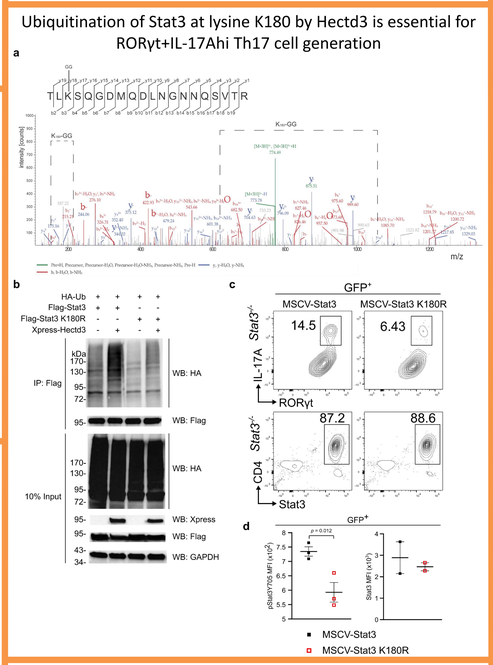
Hectd3 promotes non-degradative K27-linked polyubiquitin chains on Stat3

Hectd3 interacts with and promotes non-degradative K27- and K29-linked polyubiquitination on Malt1 and NF-κB activation in CD4+ T cells

Stat3 linker region and Hectd3 DOC domain mediate the interaction between Stat3 and Hectd3

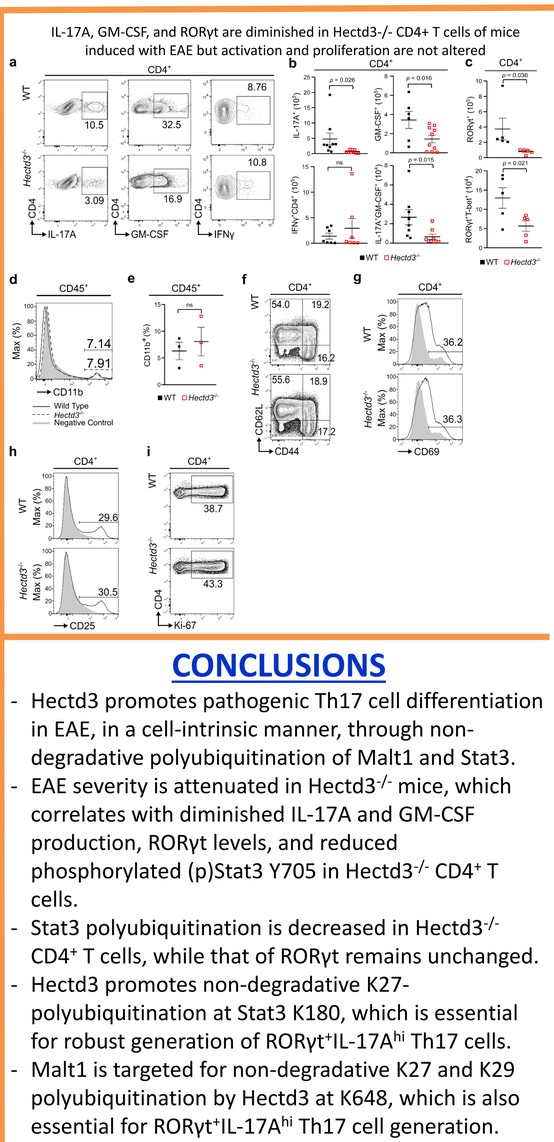


Ubiquitination of Stat3 at lysine K180 by Hectd3 is essential for RORγt+IL-17Ahi Th17 cell generation



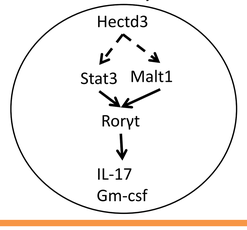
Thymic and peripheral T cell populations and polarization into Th1, Th2, and Treg conditions are normal in the absence of Hectd3

IL-17A, GM-CSF, and RORγt are diminished in Hectd3-/- CD4+ T cells of mice induced with EAE but activation and proliferation are not altered



CONCLUSIONS

- Hectd3 promotes pathogenic Th17 cell differentiation in EAE, in a cell-intrinsic manner, through non-degradative polyubiquitination of Malt1 and Stat3.
- EAE severity is attenuated in Hectd3$^{-/-}$ mice, which correlates with diminished IL-17A and GM-CSF production, RORγt levels, and reduced phosphorylated (p)Stat3 Y705 in Hectd3$^{-/-}$ CD4^{+} T cells.
- Stat3 polyubiquitination is decreased in Hectd3$^{-/-}$ CD4^{+} T cells, while that of RORγt remains unchanged.
- Hectd3 promotes non-degradative K27-polyubiquitination at Stat3 K180, which is essential for robust generation of RORγt^{+}IL-17A^{hi} Th17 cells.
- Malt1 is targeted for non-degradative K27 and K29 polyubiquitination by Hectd3 at K648, which is also essential for RORγt^{+}IL-17A^{hi} Th17 cell generation.
- Our study thus demonstrates that non-degradative polyubiquitination of Malt1 and Stat3 by Hectd3, leading to NF-kB activation and RORγt upregulation, respectively, delineates a mechanism connecting ubiquitination to Th17 differentiation and function in the context of autoimmunity and EAE



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