

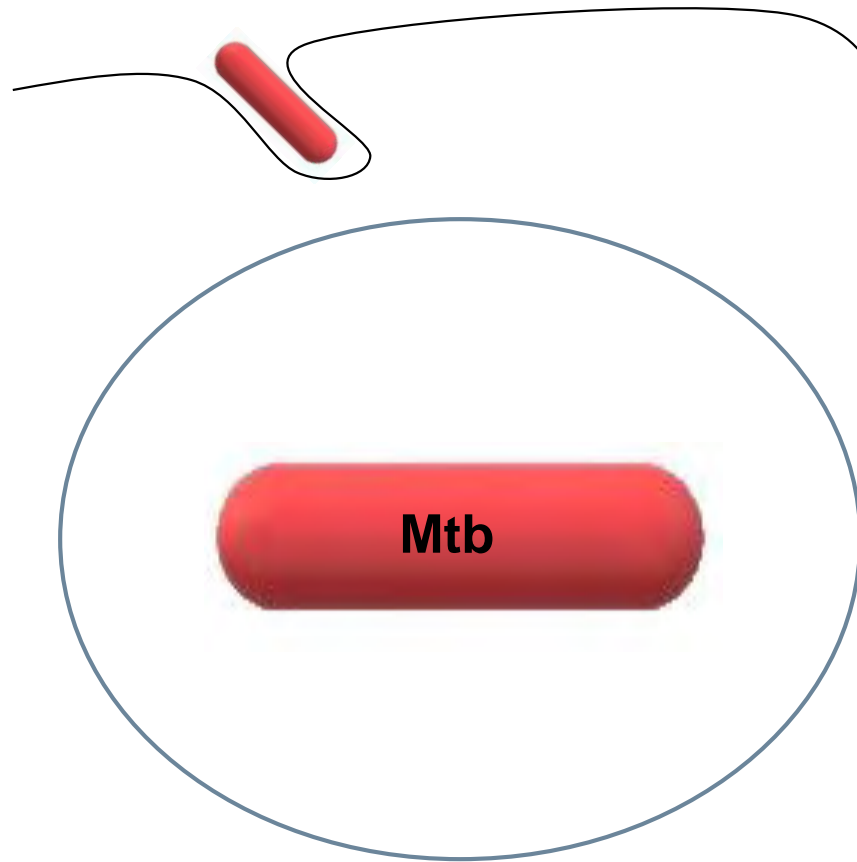
**How EsxH Controls Host
Cellular Responses to
*Mycobacterium tuberculosis***

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Mtb survives inside macrophages



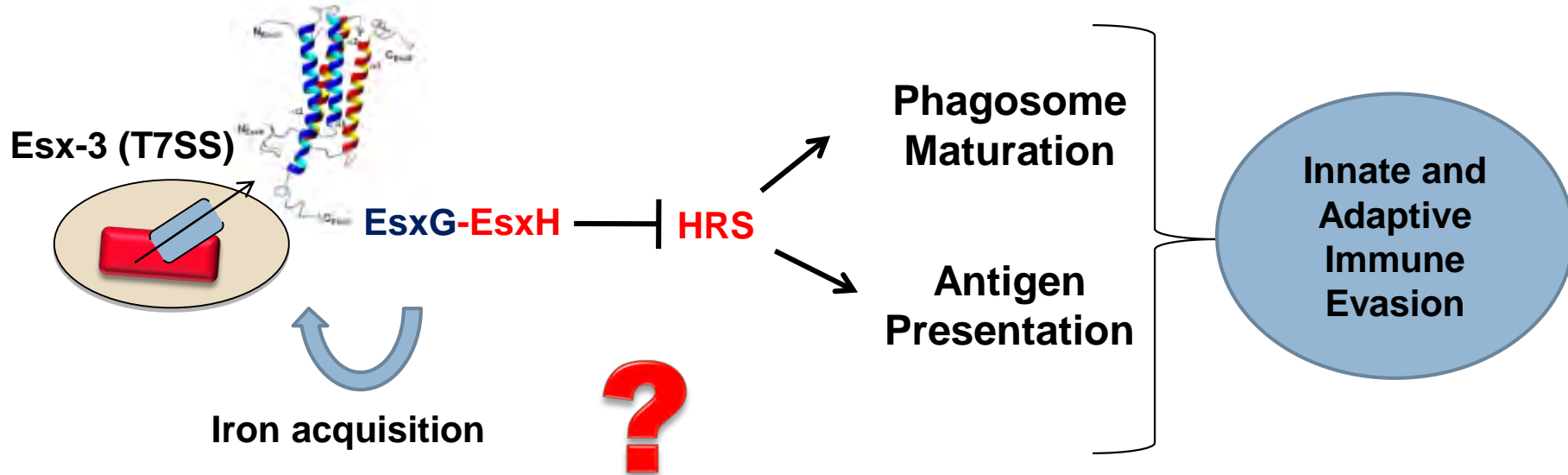
Mtb impairs lysosomal trafficking and antigen presentation.

Mtb persists despite robust CD4⁺ T cell responses.

A effective TB vaccine will have to overcome Mtb's immune evasion strategies.

Mtb virulence factor, EsxG-EsxH, is critical for immune evasion. EsxG-EsxH impairs both lysosomal trafficking and antigen presentation.

EsxG-EsxH inhibits the host factor HRS

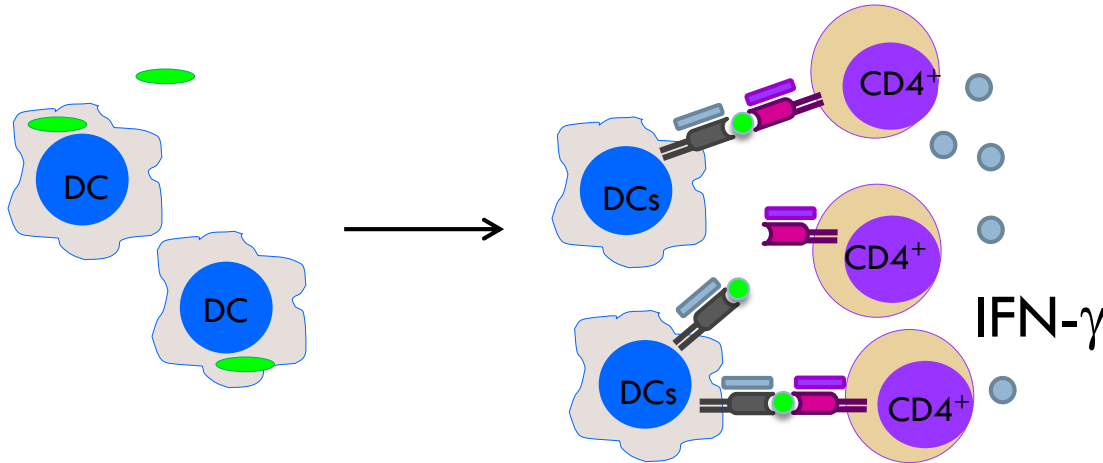


- EsxG-EsxH is critical for virulence.
- EsxG-EsxH from *Mtb* binds HRS (ESCRT-0).
- EsxG-EsxH from *Msmeg* does not bind HRS.
- By targeting HRS, EsxG-EsxH inhibits lysosomal trafficking and MHC II antigen presentation.

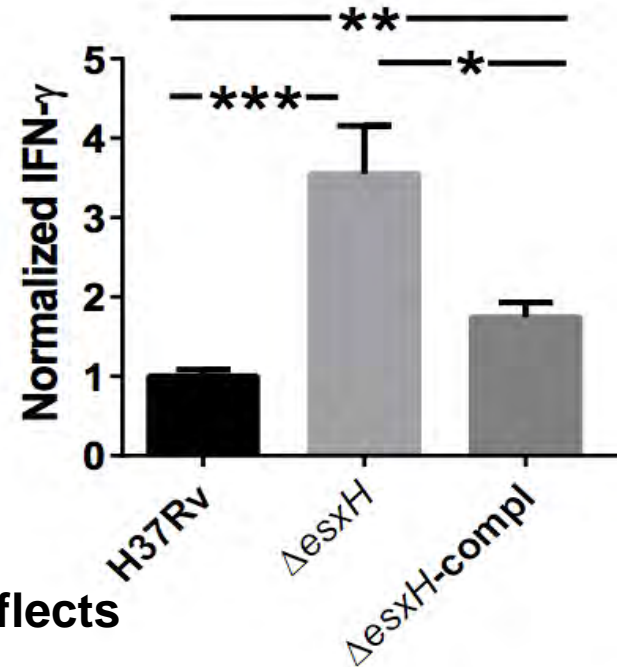
EsxH impairs antigen presentation

Dendritic cells (DCs) were infected with WT, $\Delta esxH$, or $\Delta esxH::esxH$

Infected DCs were co-cultured with CD4⁺ T cells that recognize Mtb Antigen 85B peptide.



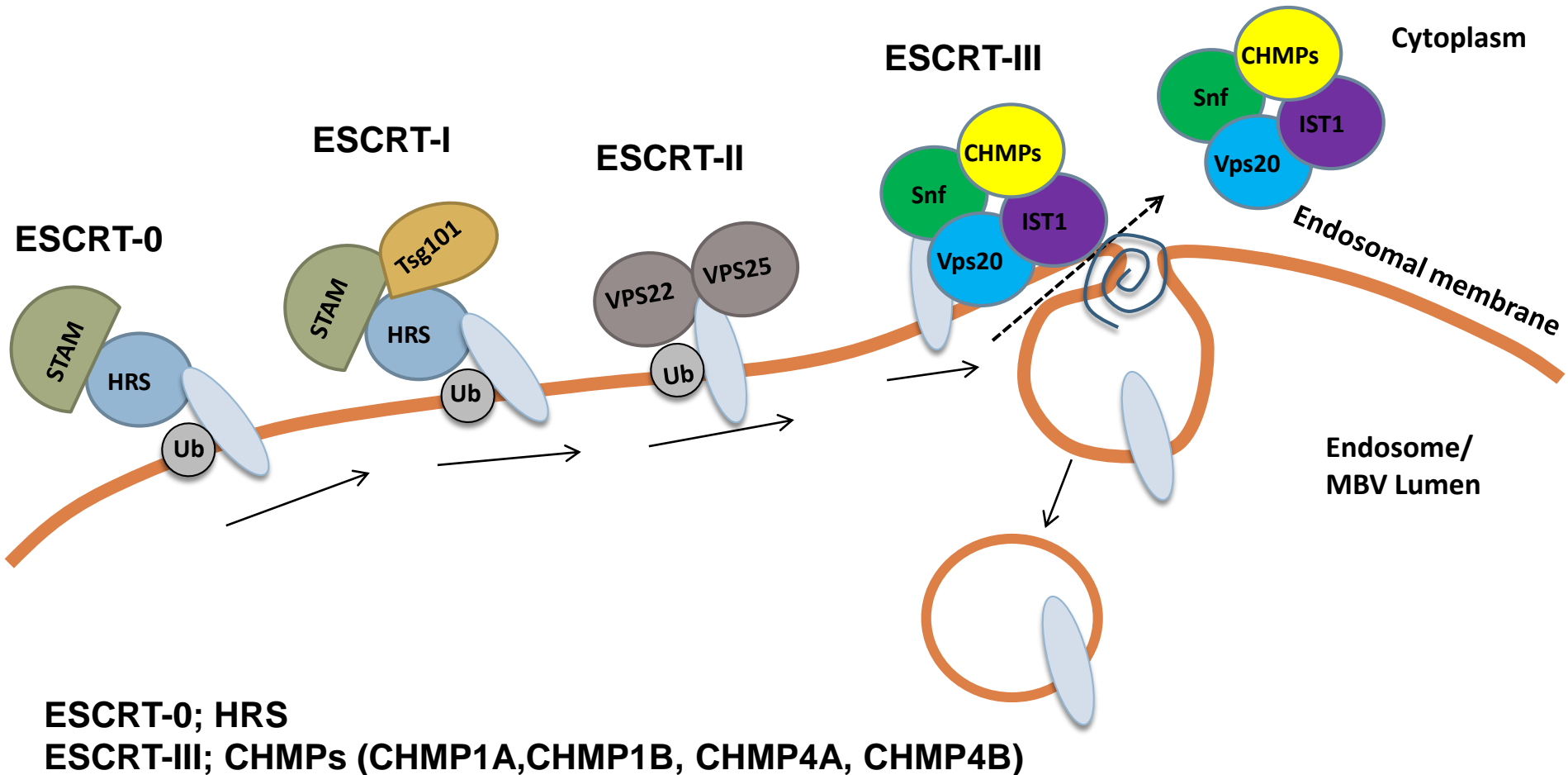
IFN- γ release by T cells reflects antigen presentation.



EsxH inhibits antigen presentation by targeting HRS/ESCRT.

ESCRT is composed of sub-complexes that traffic receptors for lysosomal degradation

ESCRT (Endosomal Sorting Complexes Required for Transport)

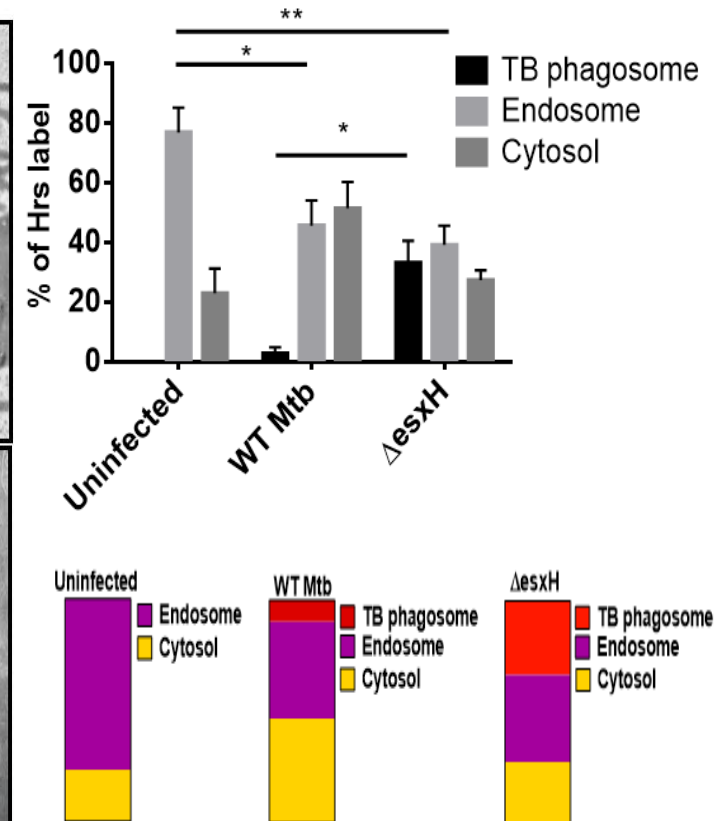
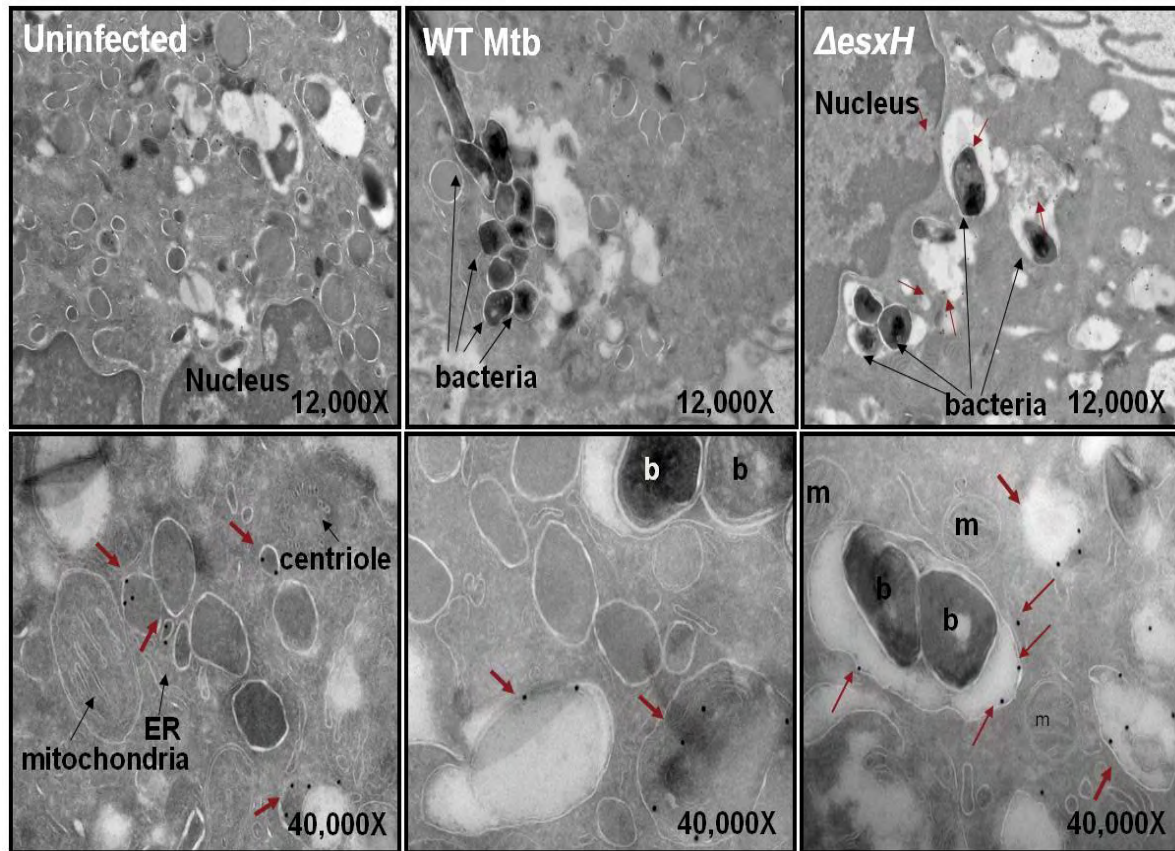


Outstanding Questions...

- **How does EsxH alters HRS/ESCRT function?**
- **Does it influence downstream ESCRT molecules?**
- **Does EsxH alter other aspects of ESCRT activity like exosome formation?**
- **Does it influence antigen transfer to uninfected cells by altering exosomes?**

EsxH prevents the recruitment of HRS to Mtb phagosomes

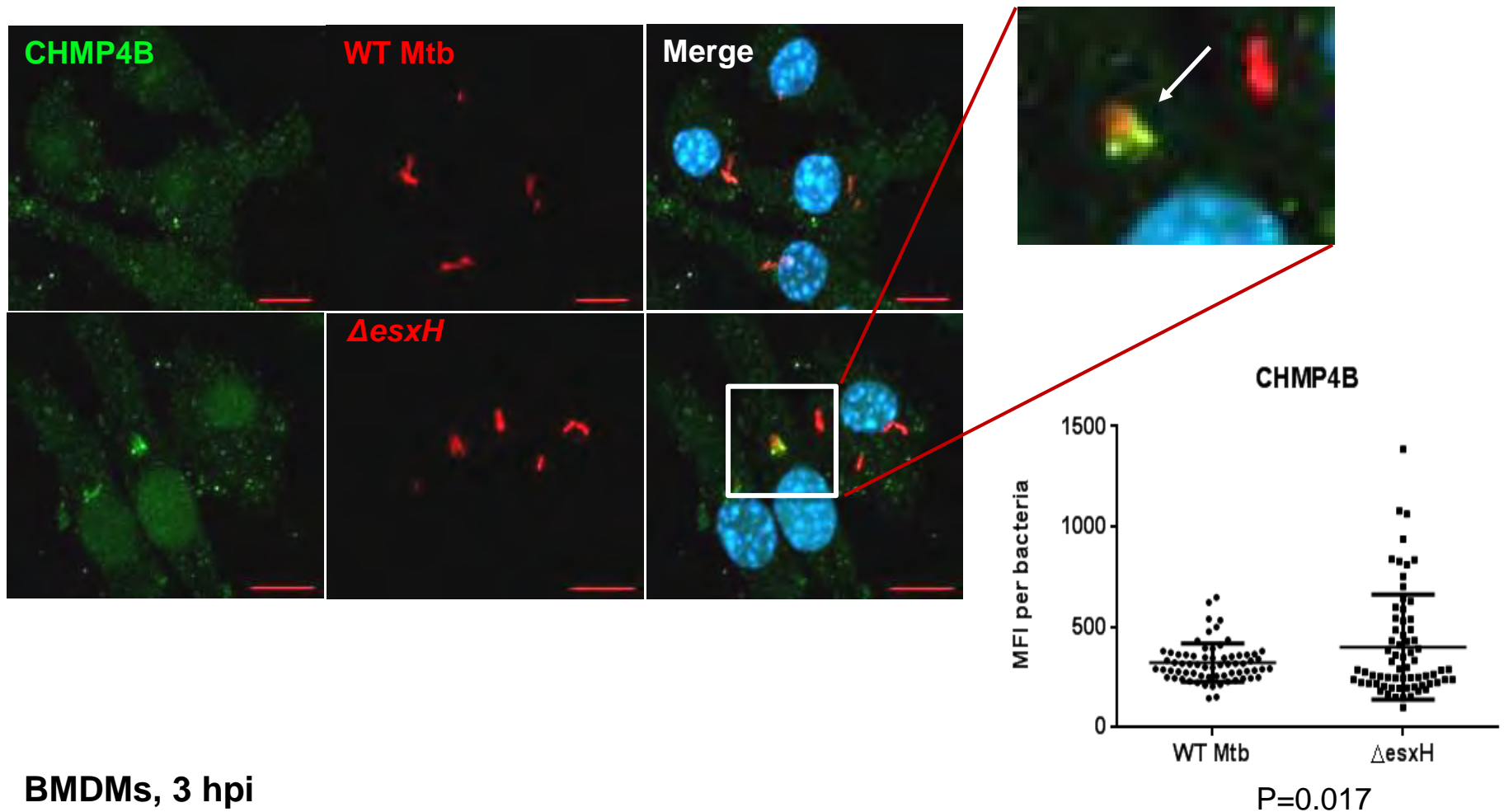
Immuno-EM of Hrs in Mtb infected BMDM



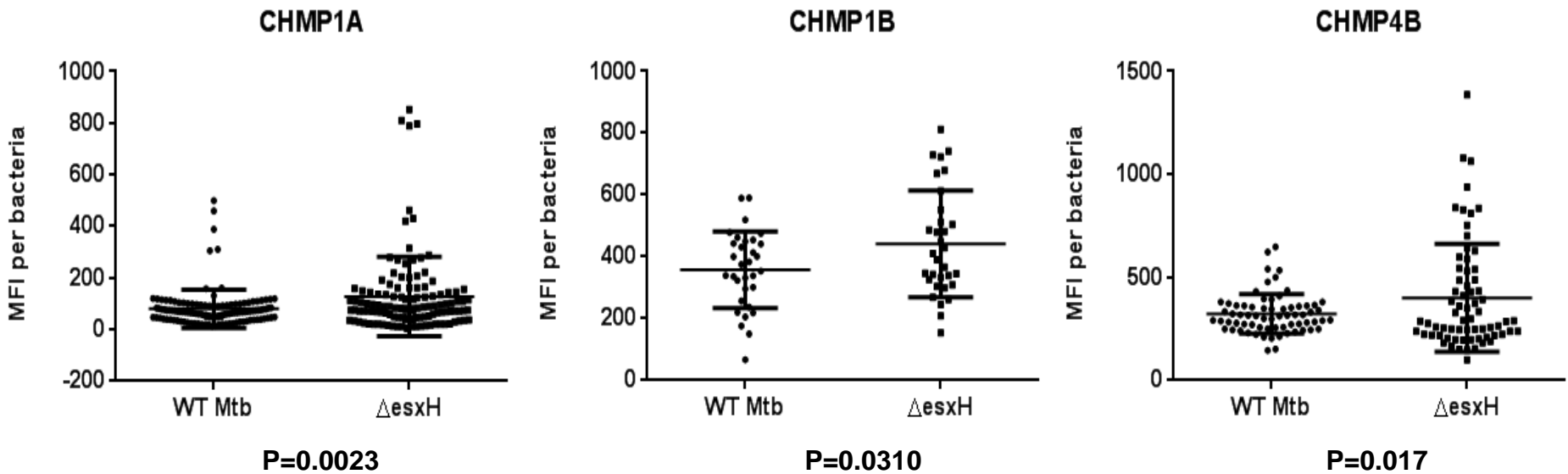
Conclusions: EsxH causes HRS to redistribute to the cytosol.
EsxH inhibits HRS recruitment to Mtb phagosomes.

EsxH antagonizes ESCRT-III recruitment

Co-localization of CHMP4B with Mtb



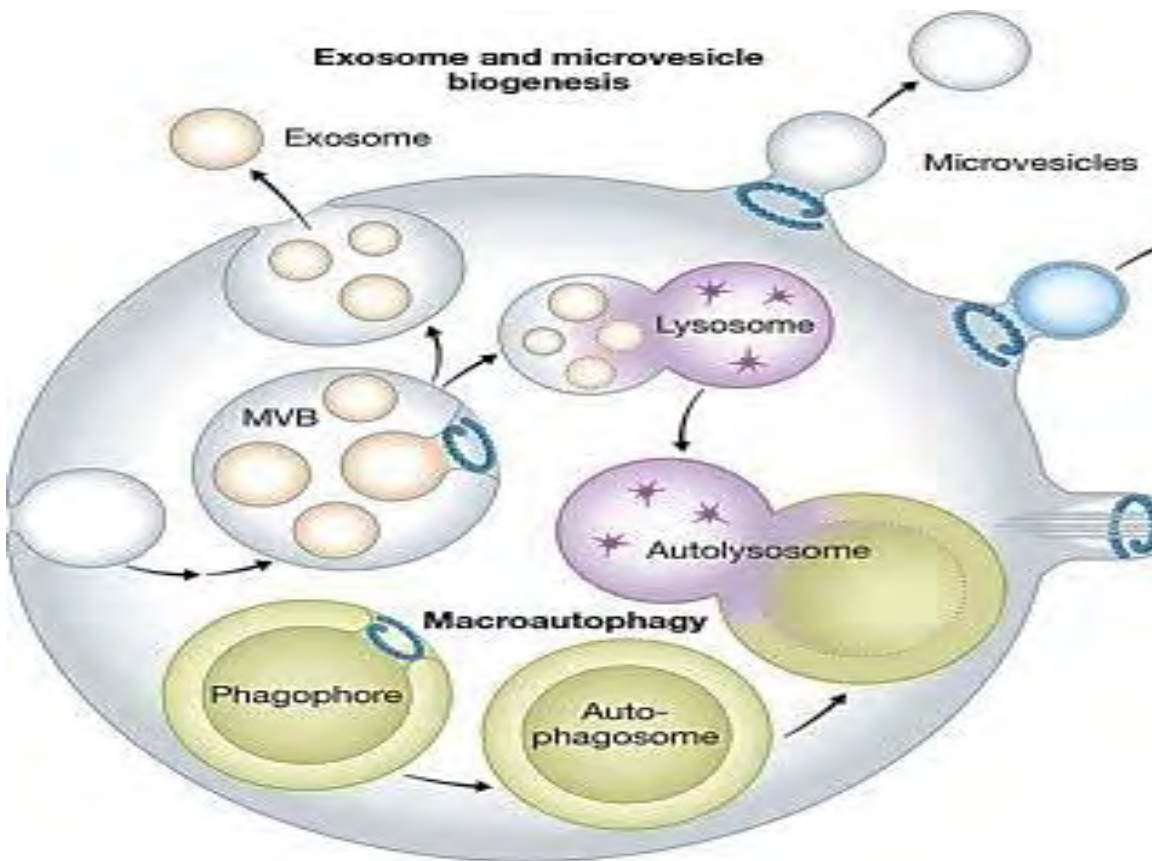
EsxH impairs ESCRT-III recruitment



Conclusion: EsxH antagonizes ESCRT-0 (HRS) and ESCRT-III recruitment to Mtb phagosomes.

Does EsxH alter exosome formation?

ESCRT is involved in exosomes and microvesicles production

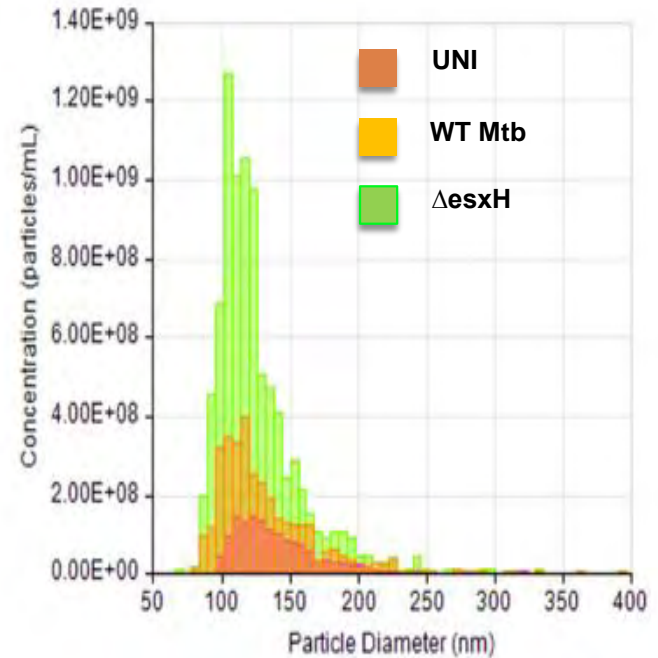
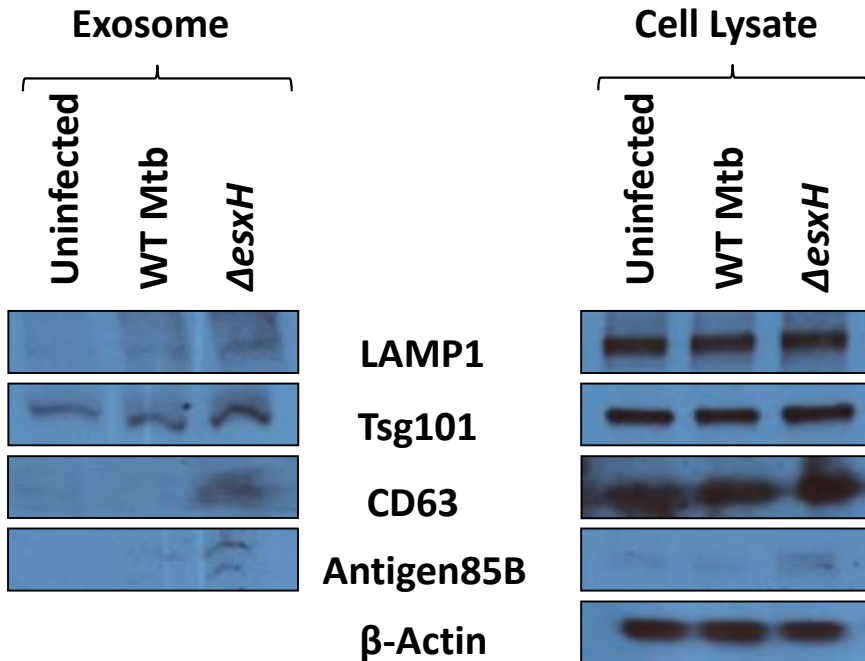
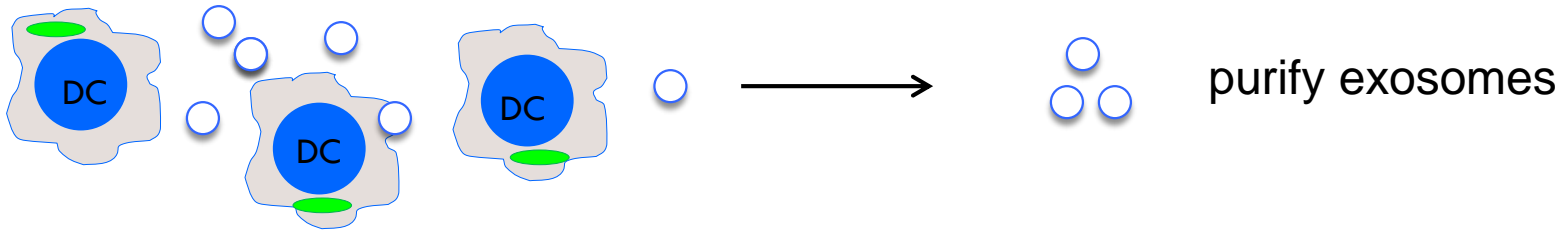


Hypothesis:

EsxG-EsxH alters exosome production by targeting ESCRT, thereby impairing antigen transfer and/or altering cytokine responses.

Does EsxH impair exosome formation?

DCs infected with Mtb, $\Delta esxH$, or $\Delta esxH::esxH$



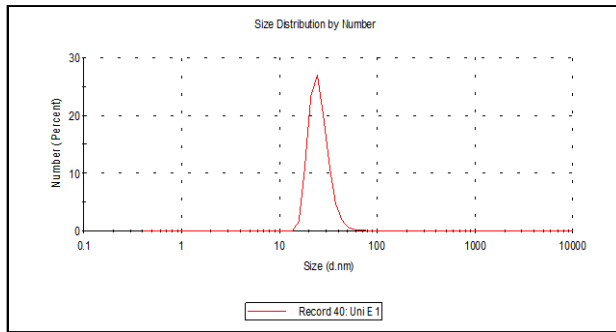
Conclusion

EsxH impairs exosome secretion, consistent with its ability to inhibit ESCRT.

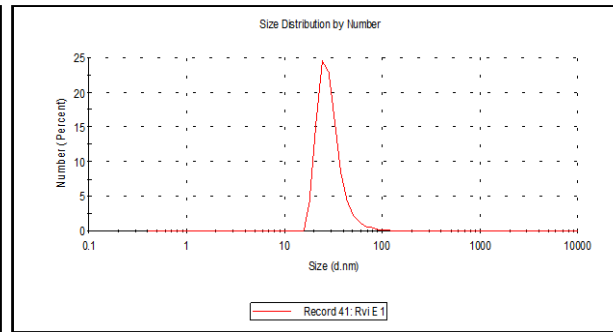
Does EsxH qualitatively alter exosomes?

Exosome Analysis: Nanosight, Size distribution by number

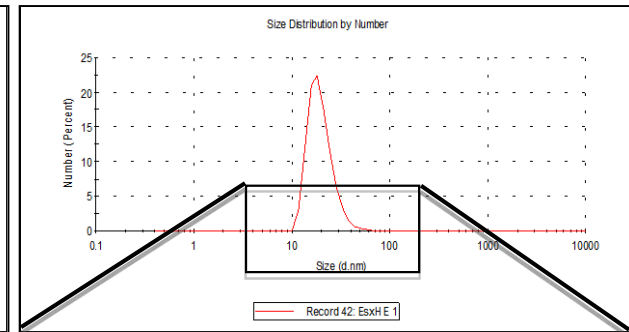
Uninfected



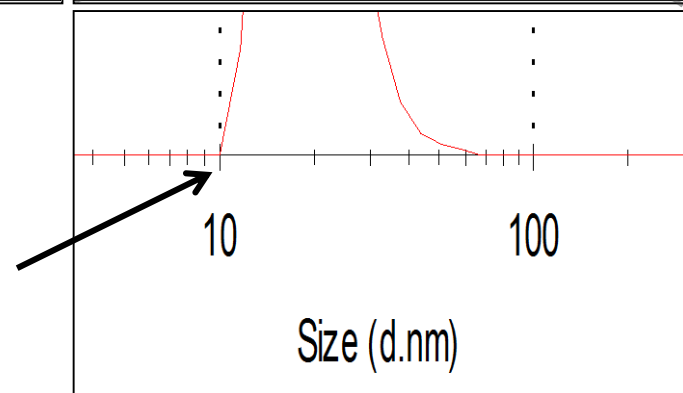
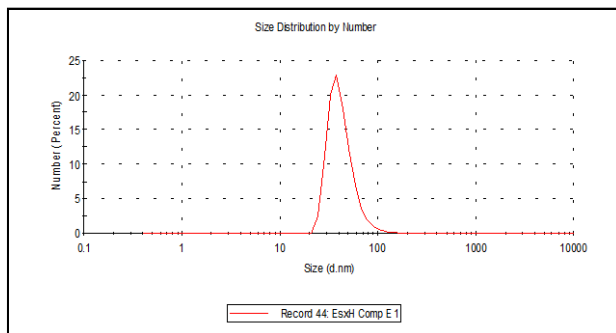
WT Mtb



Δ esxH



Δ esxH::esxH

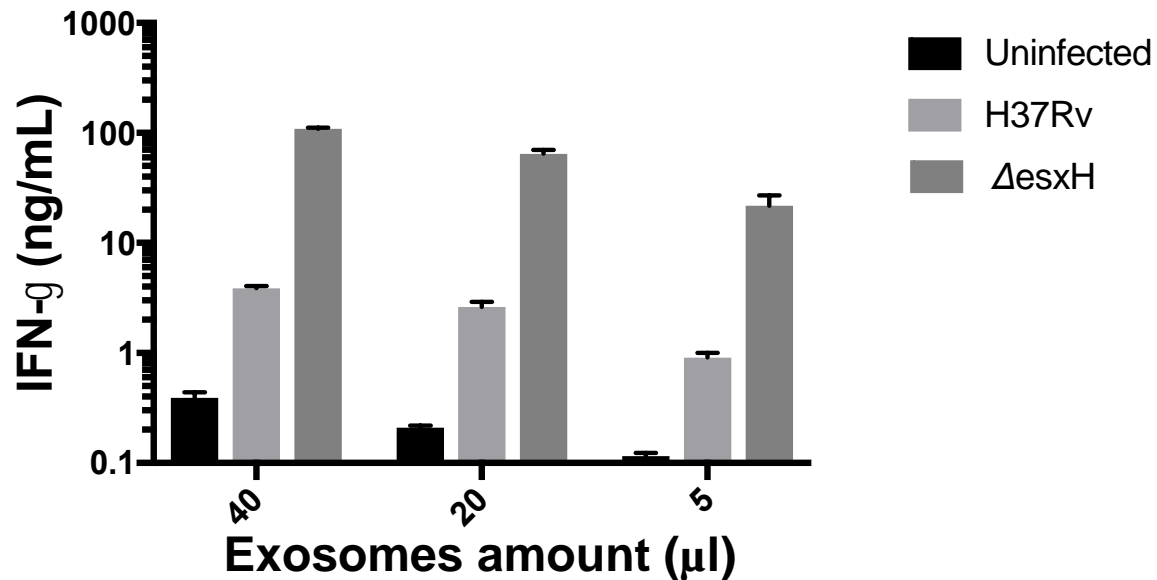
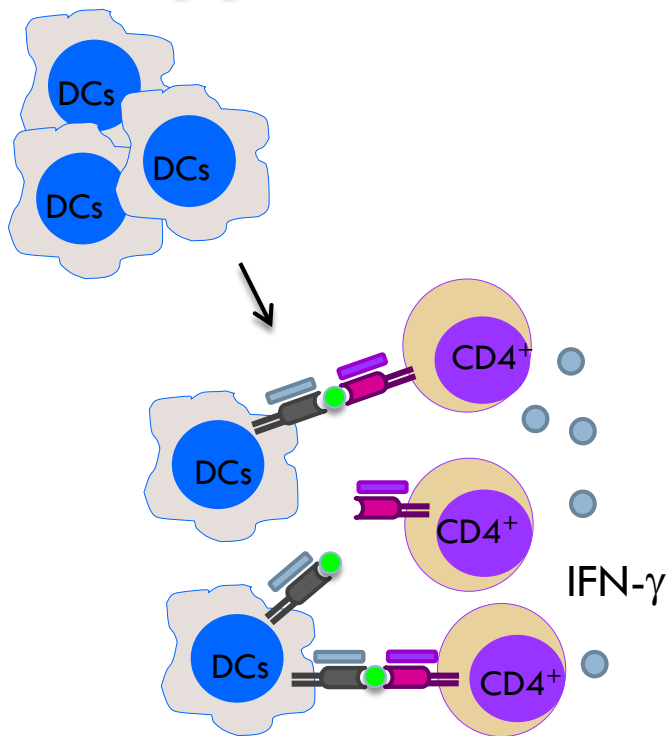


Shifted towards smaller size

Conclusion: EsxH impairs exosome secretion and changes their size distribution.

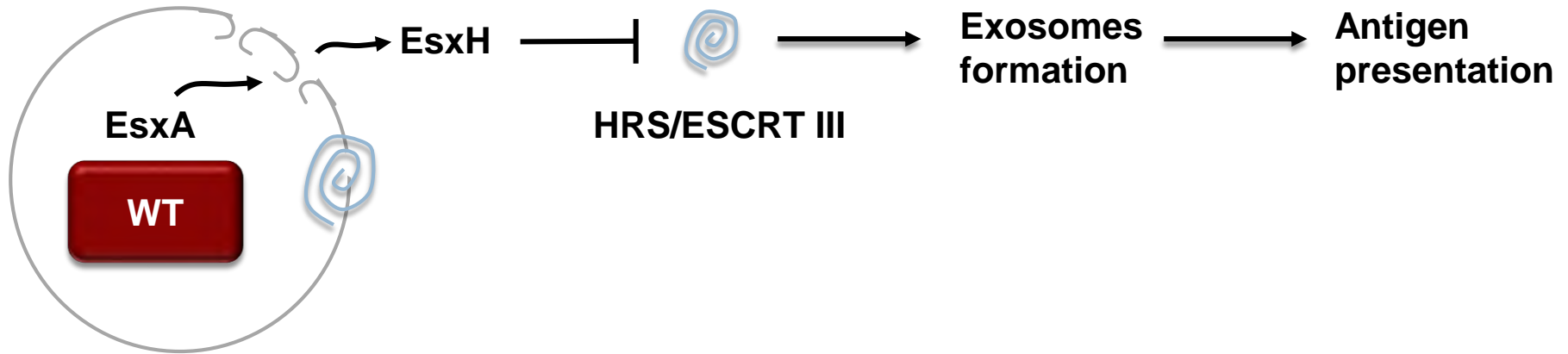
$\Delta esxH$ -infected DCs produce exosomes with enhanced ability to activate CD4⁺ T cells

- exosomes from uninfected DCs
- infected with Mtb or $\Delta esxH$



In progress: How does EsxH affect the composition of exosomes? What is the consequence of altered exosome production? Does it impair antigen transfer? Does it contribute to altered cytokine responses?

Conclusions



- **EsxH antagonizes HRS and ESCRT-III recruitment to Mtb phagosomes.**
- **EsxH impairs exosomes formation.**
- **EsxH plays a central role in Mtb evasion of innate and adaptive immunity.**
- **The ability of EsxH to block exosome production may contribute to immune evasion.**

Acknowledgements

Philips Lab

Jennifer Philips

Sandeep Upadhyay

Pallavi Chandra

Guozhe Yang

Steven Grigsby

Vinod Patil

Cynthia Portal (NYU)

Wandy Beatty

EM Facility, WashU

William Jacobs, Jr.
(AECOM)

Joann Tufariello
(Georgia State U)

Nano Research Facility, WashU



This work is supported by WashU, St. Louis and NIH grant (R01 AI087682) to Dr. Jennifer Philips.

Thank you