

How does LAG3 work

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UPMC Hillman Cancer Center*

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University of
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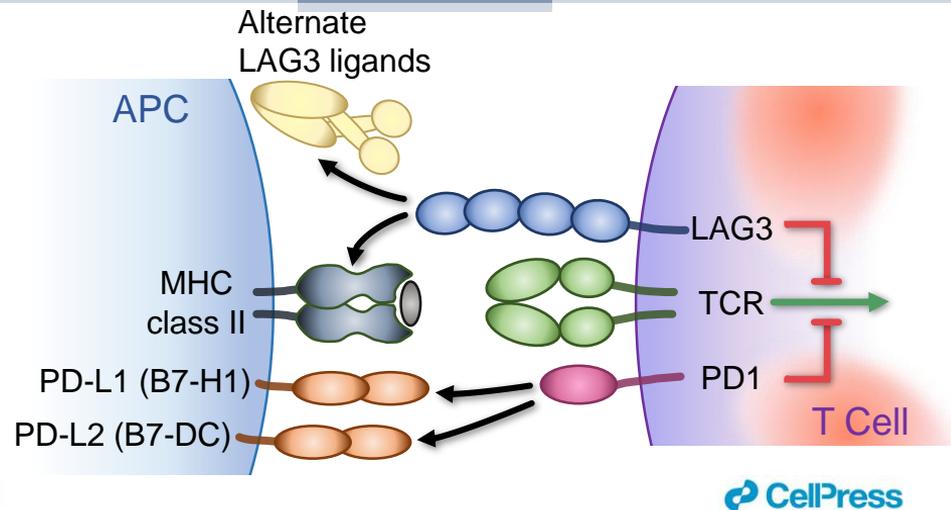
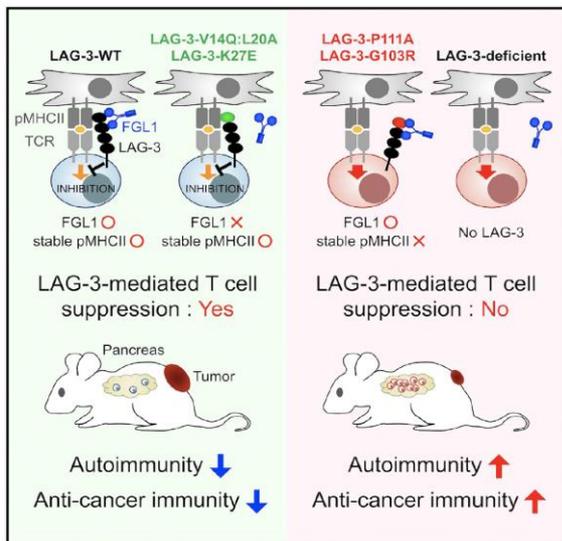
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Disclosures

- Patents:** LAG-3 (BMS), Nrp1 and IL-35: patents granted and pending.
- Founder:** Potenza Therapeutics, Tizona Therapeutics, Trishula Therapeutics, Novasenta.
- Stock Owner:** Potenza Therapeutics, Tizona Therapeutics, Trishula Therapeutics, Novasenta, Oncorus, Werewolf, Apeximmune.
- SAB:** Tizona, Werewolf, F-Star, Bicara, Apeximmune.
- Consultation:** Potenza/Astellas, BMS, MPM, Oncorus, Incyte, Almirall, G1 Therapeutics, T7/Imreg Bio, Inzen Therapeutics.
- Grants/SRAs:** Potenza/Astellas, BMS, Novasenta.

LAG3 - the third checkpoint

- LAG3 limits T cell function and homeostasis
- LAG3 impacts TCR signaling, like PD1, but with a distinct mode of action
- LAG3 binds to MHC class II, but may have other ligands (eg. FGL1; *Cell* 2018)



Immunity

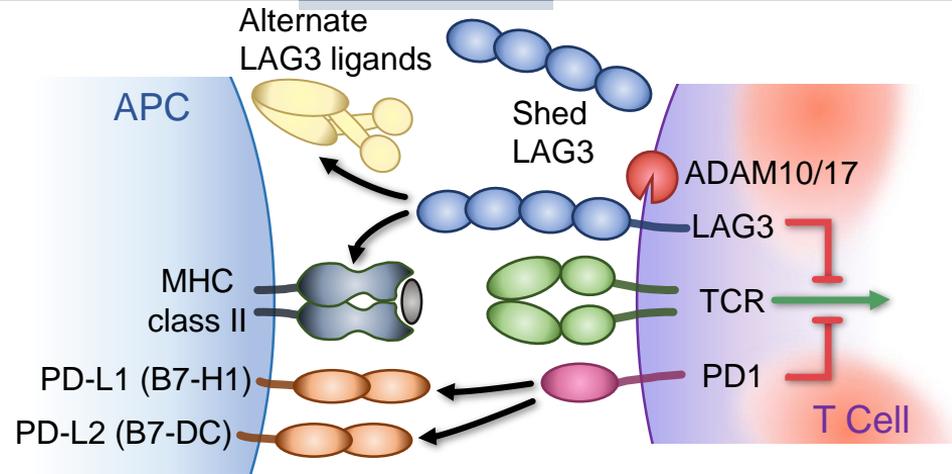
Article

Binding of LAG-3 to stable peptide-MHC class II limits T cell function and suppresses autoimmunity and anti-cancer immunity

Takumi Maruhashi,¹ Daisuke Sugiura,¹ Ii-mi Okazaki,^{1,2} Kenji Shimizu,¹ Takeo K. Maeda,² Jun Ikubo,² Harunori Yoshikawa,³ Katsumi Maenaka,⁴ Naozumi Ishimaru,⁵ Hidetaka Kosako,³ Tatsuya Takemoto,⁶ and Taku Okazaki^{1,2,7,*}

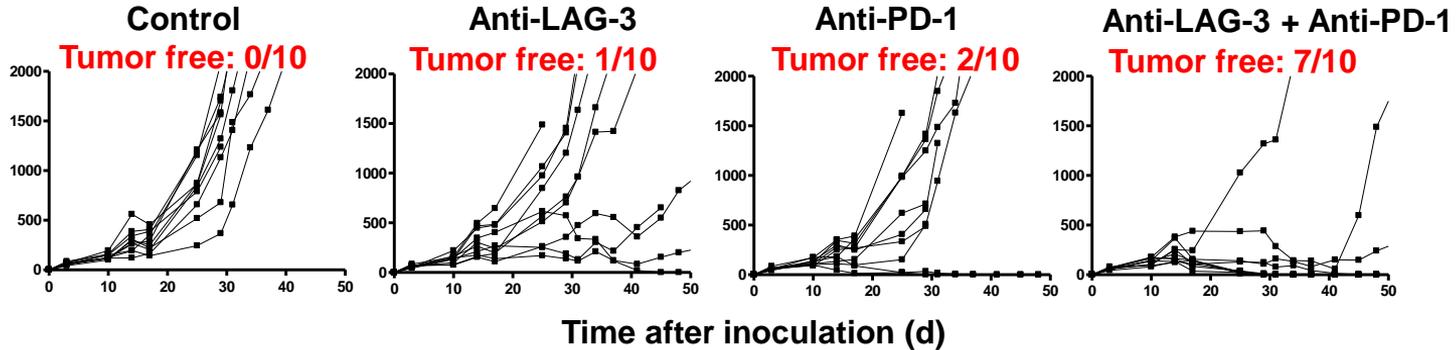
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- Anti-LAG-3 / anti-PD-1 exhibit synergistic combinatorial anti-tumor activity (*CR* 2012)

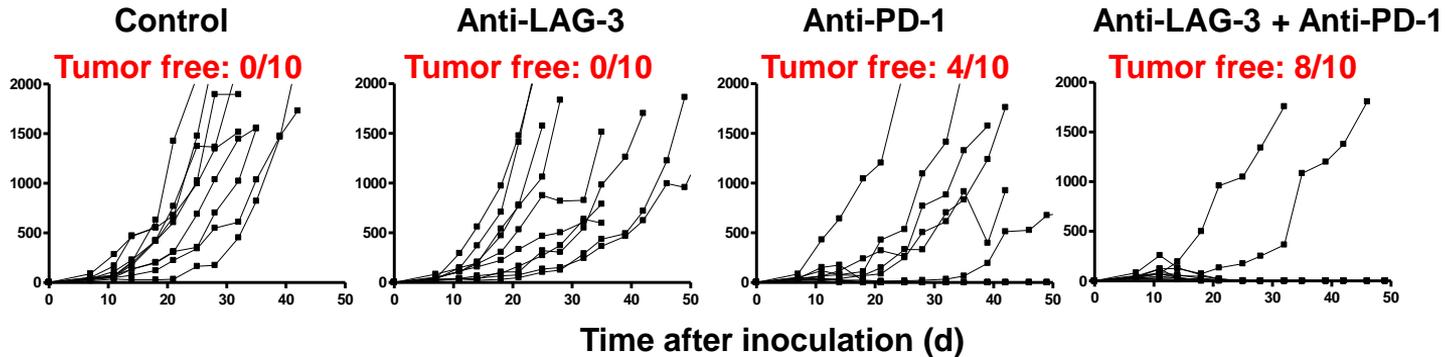


Tumor clearance with combinatorial anti-LAG-3 / anti-PD-1 treatment

Sa1N Fibrosarcoma - A/J mice (Ab treatment = 10mg/kg at day 8, 11, 14)

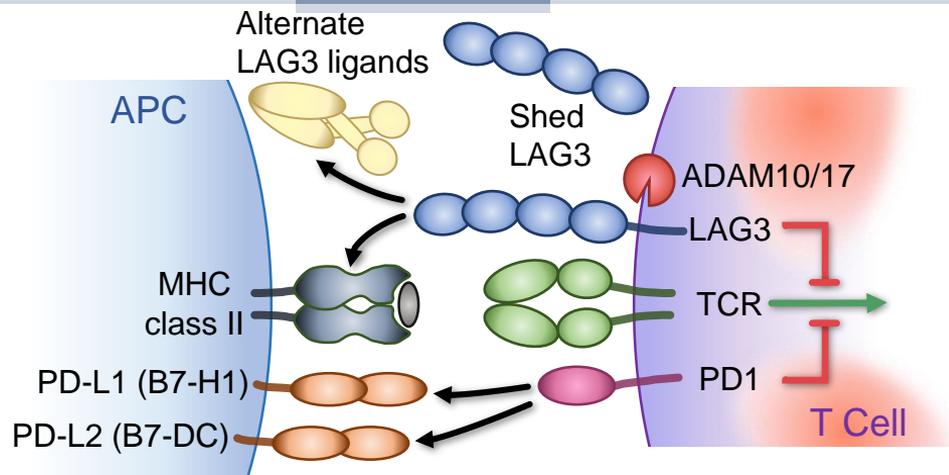


MC38 Adenocarcinoma – C57BL/6 mice (Ab treatment = 10mg/kg at day 8, 11, 14)



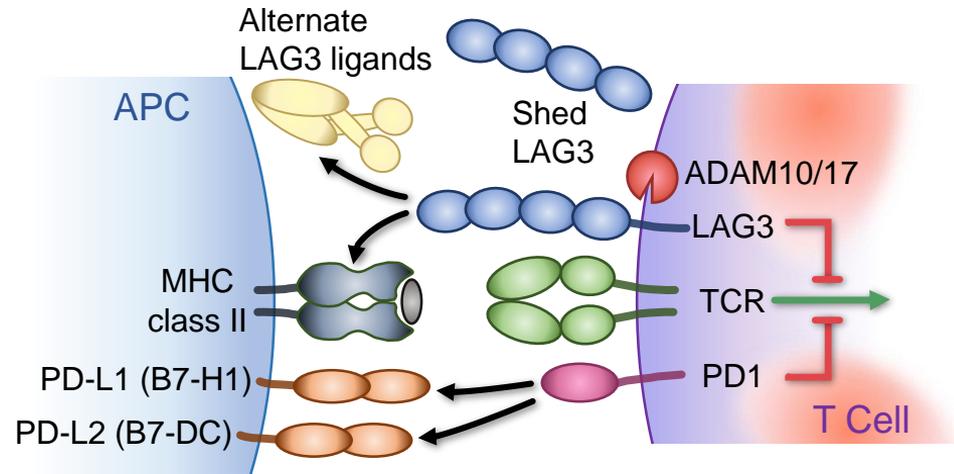
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- Anti-LAG-3 / anti-PD-1 exhibit synergistic combinatorial anti-tumor activity (*CR* 2012)
- Relatlimab (BMS) first-in-clinic: currently over 20 LAG3 targeting therapeutics in clinical trials!
- REALTIVITY-047: Rela + Nivo phase 2/3 trial in treatment-naive patients with metastatic melanoma met primary endpoint of progression-free survival (*Tawbi, 2022, NEJM*)
- March 2022: FDA approval of a fixed dose dual immunotherapy combination of Rela+Nivo (Opdualag) for the treatment of unresectable or metastatic melanoma



How does LAG3 work?

- How does LAG3 mediate its inhibitory activity?
- Is MHC class II ligand binding required for LAG3 function?



Cliff Guy



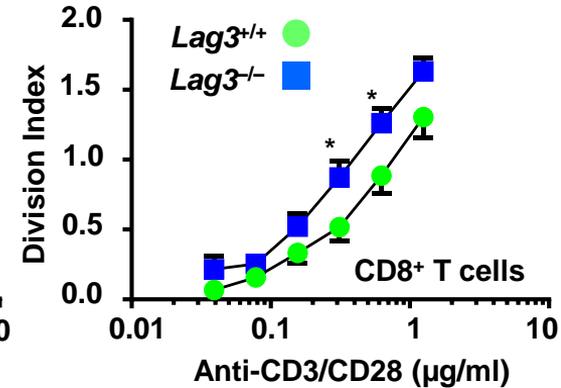
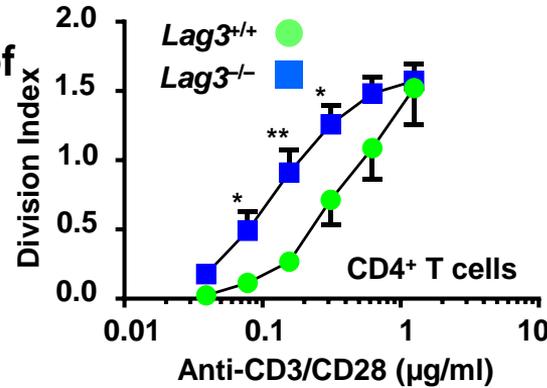
Creg Workman

Curious Observations:

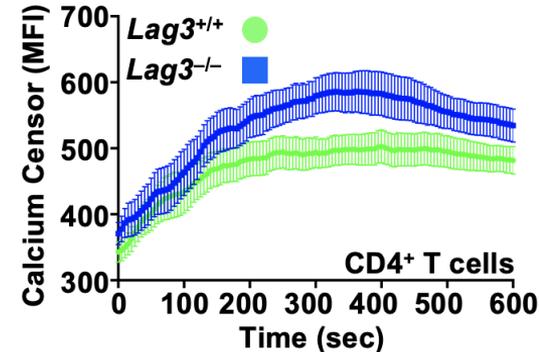
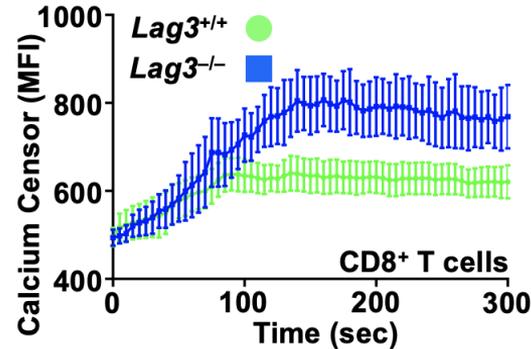
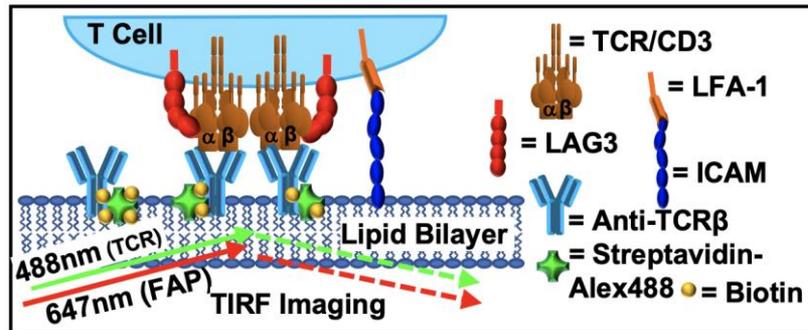
- Our anti-mouse LAG3 blocking mAb (C9B7W) does not block LAG3:MHC class II interaction
- LAG3 inhibitory activity cannot be induced by receptor crosslinking
- LAG3 function is co-receptor (CD4/CD8) dependent (*J I 169:5392, 2002*)

LAG3 can function in the absence of MHC class II

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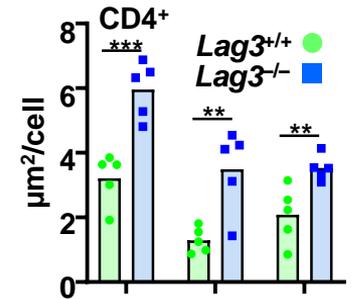
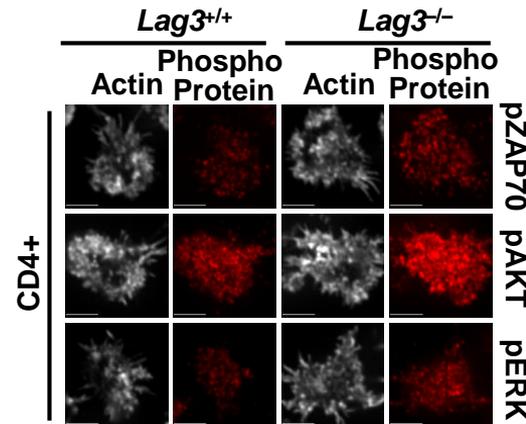
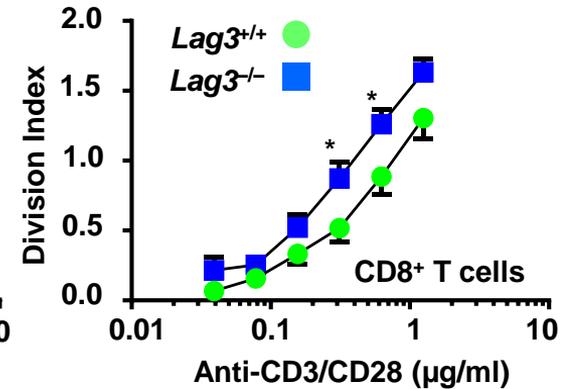
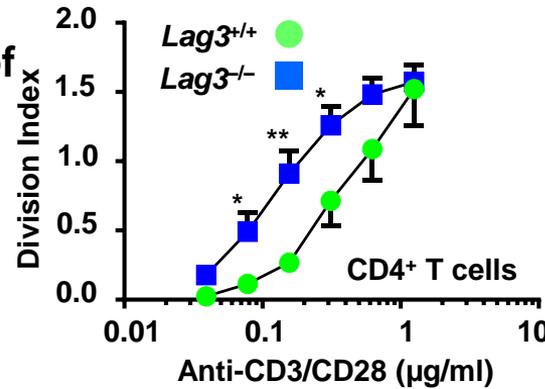


Total Internal Reflection Fluorescence (TIRF) Microscopy



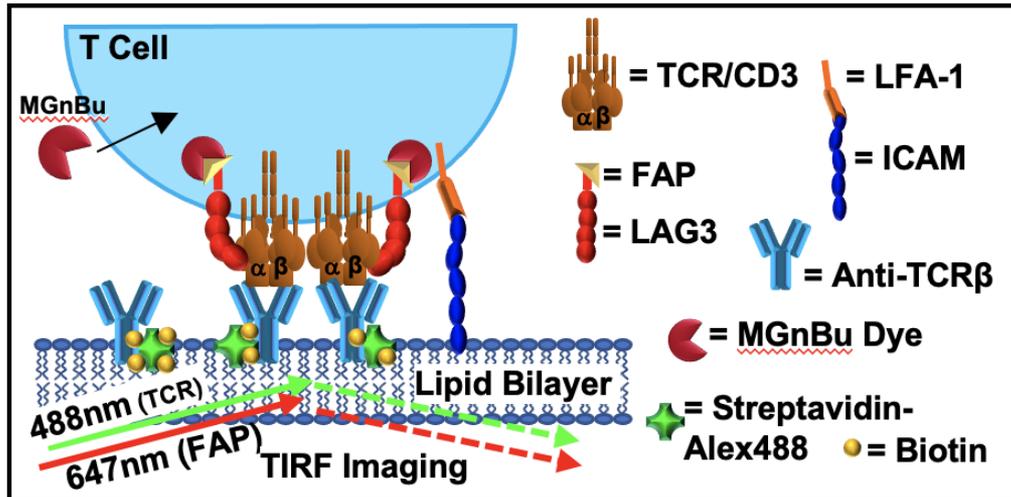
LAG3 can function in the absence of MHC class II

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LAG3 associates with TCR/CD3 complex

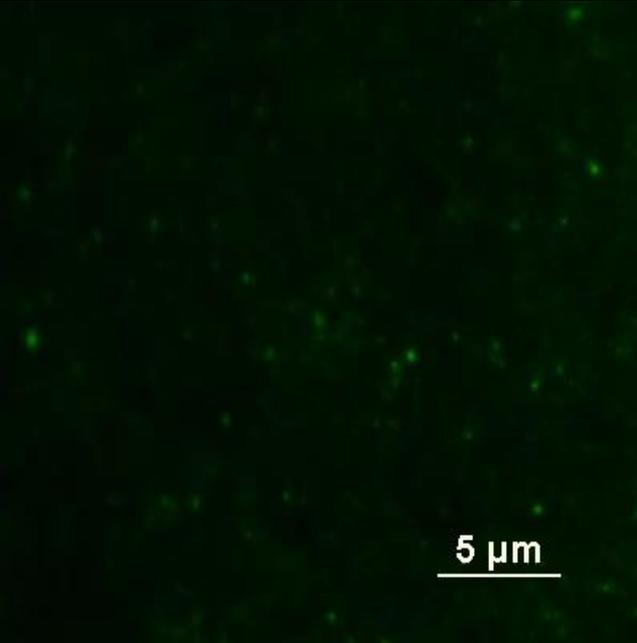
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~13:1 (TIRF, STED, STORM, Expansion Microscopy, Co-IP) [**ligand in cis**]



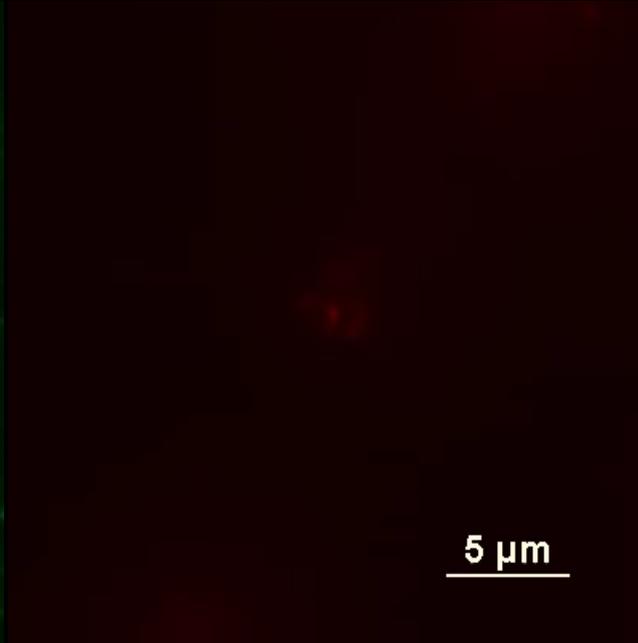
Total Internal Reflection
Fluorescence
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LAG3 tracks with the TCR into the IS

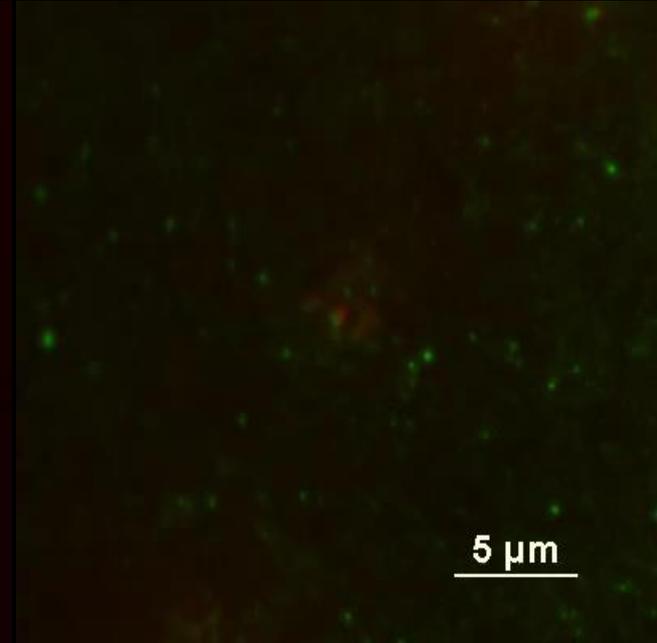
TCR



LAG3

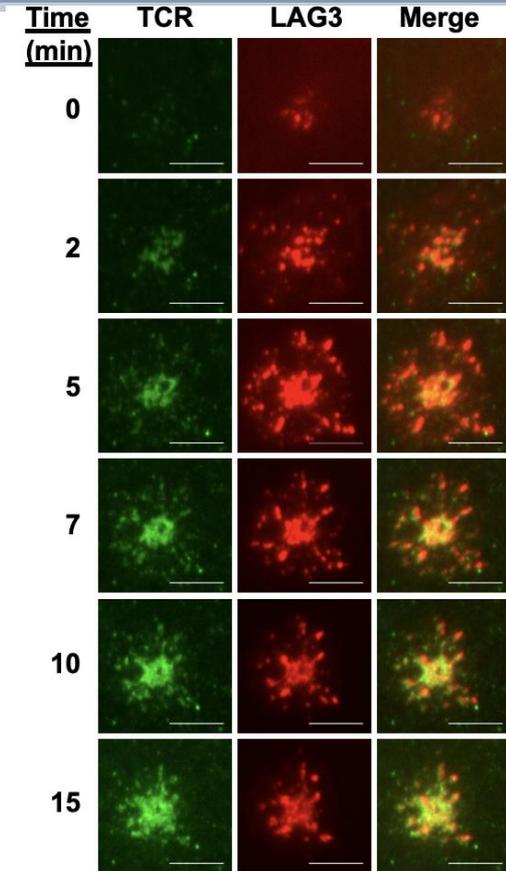
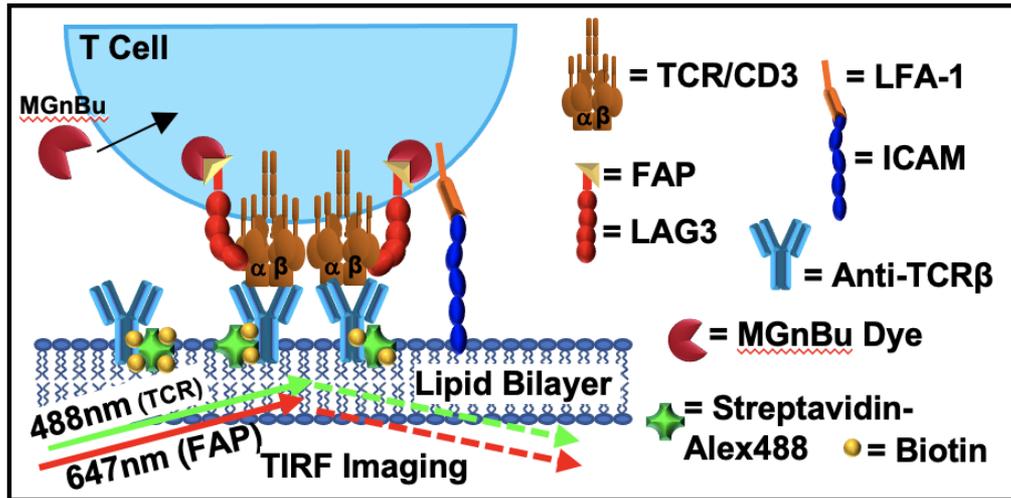


TCR + LAG3



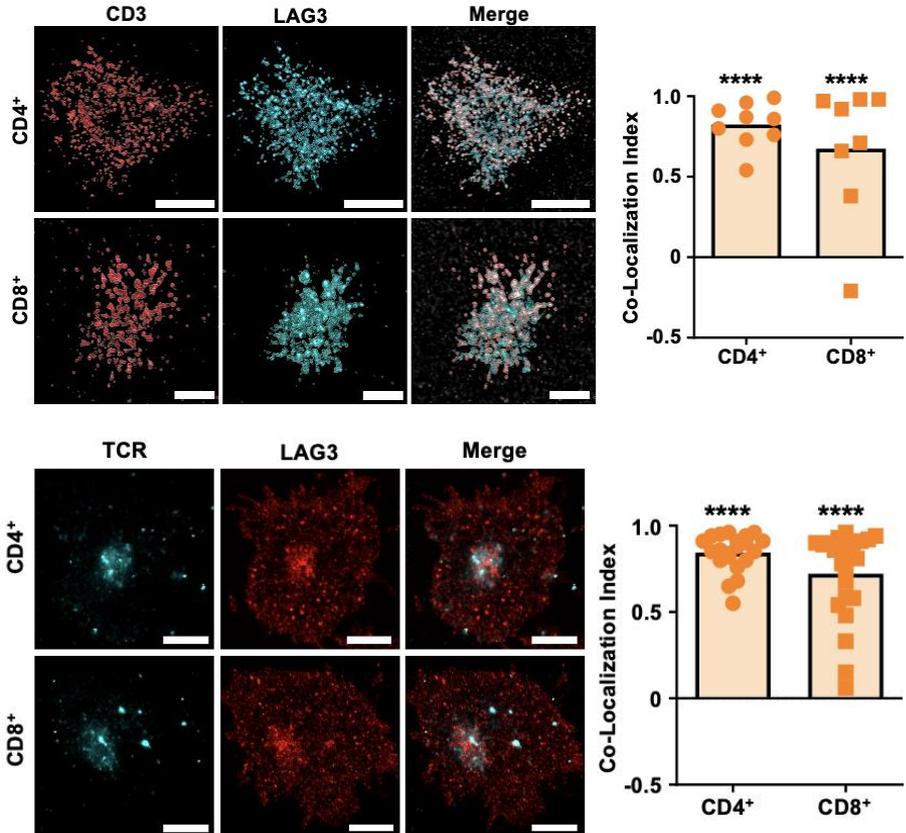
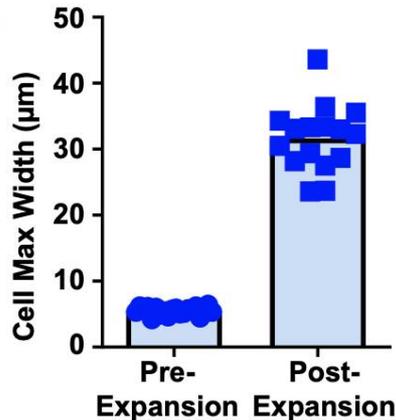
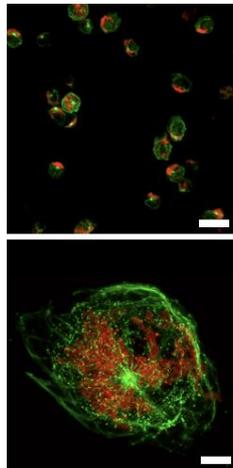
LAG3 associates with TCR/CD3 complex

- LAG3 can function in the absence of MHC class II
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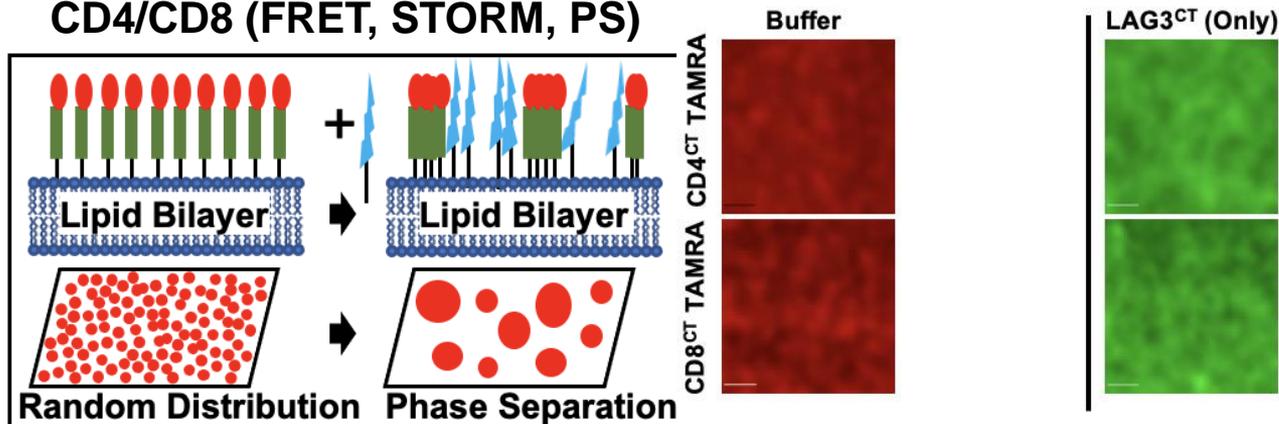
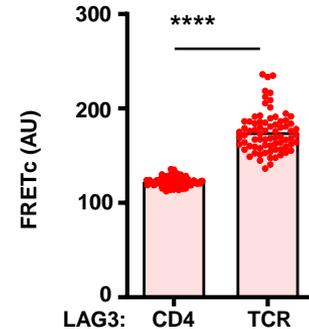
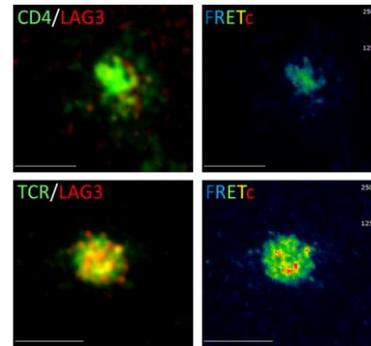
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LAG3 also closely associates with CD4/CD8

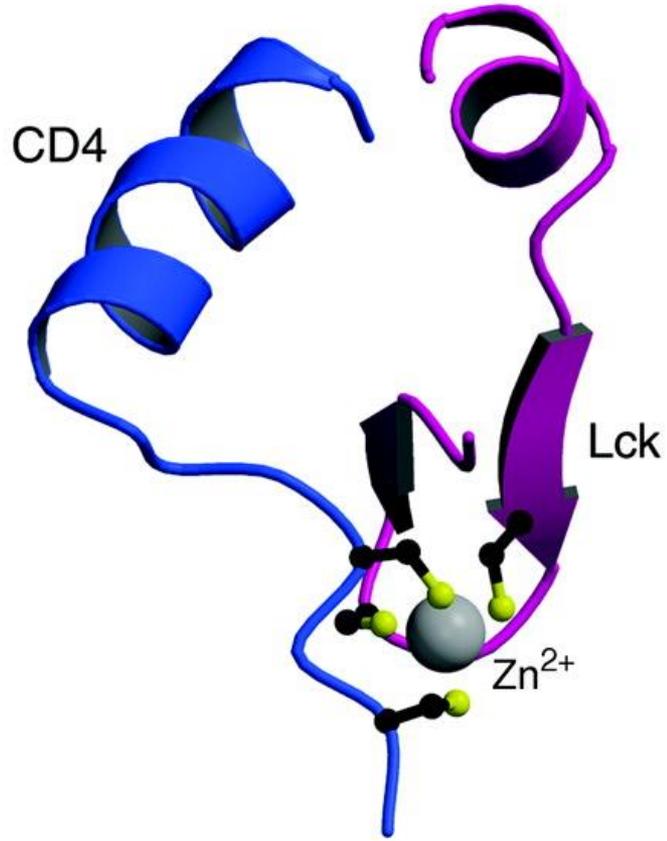
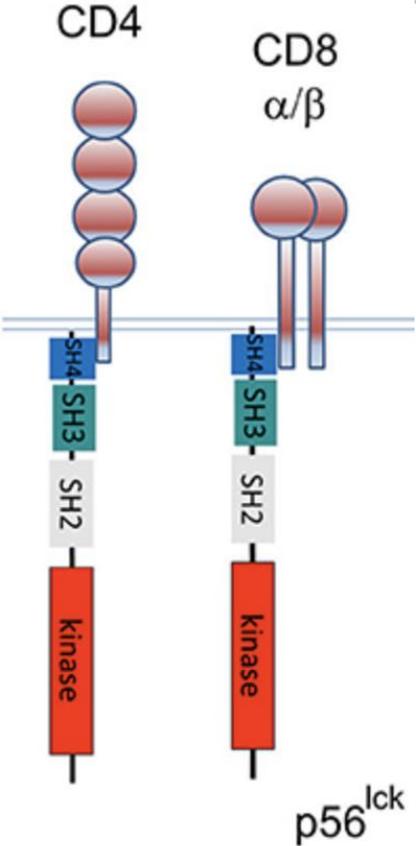
- LAG3 can function in the absence of MHC class II
- LAG3 associates with TCR/CD3 complex: ~13:1 (TIRF, STED, STORM, Expansion Microscopy, Co-IP) [ligand in cis]
- LAG3 also closely associates with CD4/CD8 (FRET, STORM, PS)



Phylogenetically conserved LAG3 repetitive 'EP' motif

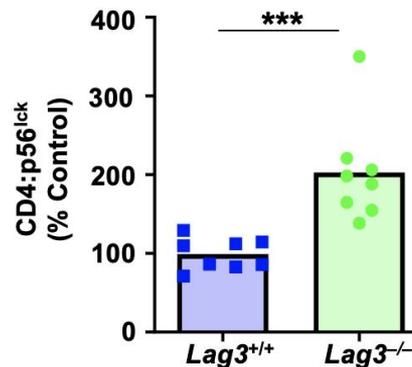
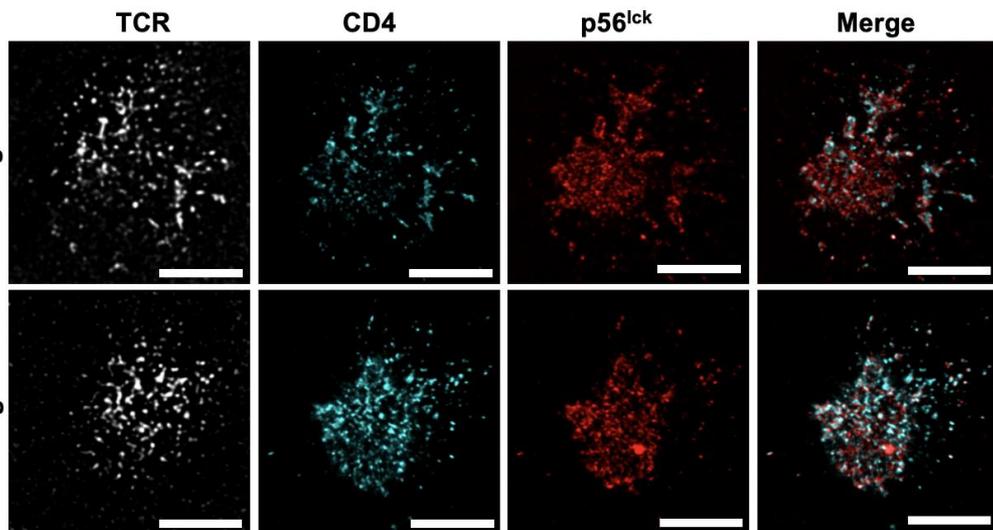
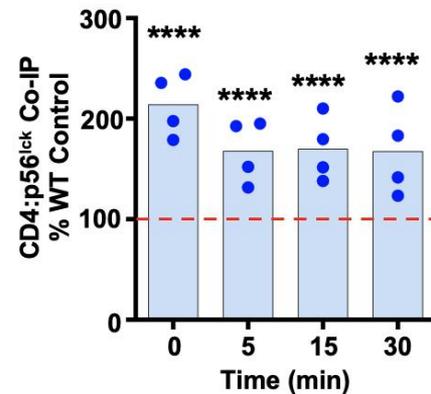
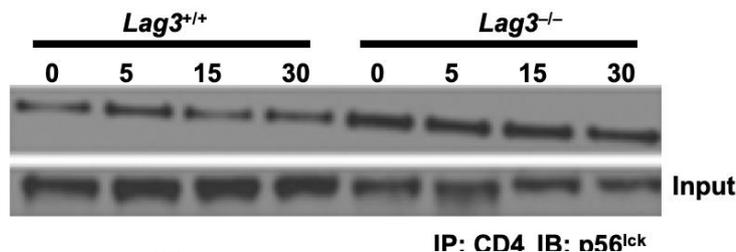
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Rat	RRQLLRRRFSALEHGIRPPPVSQSKI	EELEREPETEMEPEPEPEPDPEPQPEPELEPESRQL
Human	RRQWRPRRFSALEQGIHPRQAQSKI	EELEQEPEPEPEPEPEPEPEPEPEQL .
Gorilla	RRQWRPRRFSALEQGIHPPQAQSKI	EELEQEPEPEPEPEPEPEPEPEPEQL .
Chimpanzee	FSQWRPRRFSALEQGIHPPQAQSKI	EELEQEPELEPEPEPEPELGPPEPEPEQL
Orangutan	RRQWRPRRFSALEQGIHPPQAQSKI	EELEQEPELEPEPEPEPEPEPQPEPEQL
Gibbon	RRQWRPRRFSALEQGIHPPQAQSKI	EELEQEPEPEPEPEPEPEPELGPPEPKPEQL
Macaque	RRQWRPRRFSALEQGIHPPQAQSKI	EELEQEPELEPEPEPELERELGPPEPEPGPEPEPEQL
Marmoset	RRQWRPRRFSALEQGIHPPQAQSKI	EELEQELEPEPEPEPEPEPEPERAPEPGPEQL
Bushbaby	KRPWRPRRFSALEHGIHSPQAESKI	EGDQEPDLEPEPELDPEIGPELEPGLDPELEPELALAEQL
Mouse Lemur	RRPWRPRRFSALEDGIHPPHAESKI	EGLEQELEPEPELEQEPELGLELEQL
Panda	RRQWRPRRFSALEHGTHPPQAQSKI	GELEQEPELEPEPELELEVEPESELEPELEPEPEPE
Elephant	RRPWRPRRFSALENGIHPPQAQSKT	EELELEPEQEMEPEPELELELESEPE
Horse	RRQWRARRFSALEHGIHPPQAQSKI	EELEPEAQPETELALEPDPELELEQP
Cow	RRQW-PRRFSALEHGTHPSQASSKT	GELEPELEPEPDPEVEPEPEPEPESQPPQLQPEQP
Pig	RRRWRPRRFSALEHGTHPPQAQSKT	GELEPEPELEPEPELEVEPQPEQP
Dog	GLKWRPRRFSALELGTHPPQAQSKI	GELEQEPELELEPEPELEPEPEPEEL
Cat	RRQWRPRRFSALEHEIHPPQTQSKI	GELEPEPELEPEPEPEPEPEPEQL
Guinea Pig	KRQWRSSRFSALEFGIRPPQAQSKI	EEVEQEADLETETPQSCSLGPQQPPSPFFHHCAGC
Kangaroo Rat	RRQWRPRRFSALELGTYPPQAQSKT	EEWELDMEPEMEQELEPPTEPELTQL
Pika	RRQWRPRRFSALEHGAPPPHAQSKT	EELEPEELQPEPEPEPELGLPEPEPRQL
Rabbit	RRQWRPRRFSALEHGAPPPQAQSKI	AASSVSPSPSPEESLLPGCVKPSPLPSAALPPTGCQL
Squirrel	RRQWRPRRFSALEHGIHPPQSQSKI	EEPEQEPEPEPEPEPEQEPEPELELL
Shrew	RRQWRPRRFSALEQGVHPPQAQSKI	EELEQDPELEPGTEPEPEPELEPAPELEQSR
Tree Shrew	RRRWRPRRFSALEHGIDPPQAQGKI	EELEQGLELEPEPEPGPEPGPEPEHF .
Wallaby	RPIQLPRRFSALECAAQSSHGQNKI	EEMEREVSGLEPHQELKMGQL .
Tasmanian Devil	RQGQFLRSFSALEDAAQNPQRQSKI	EEMEPECPCQS .
Megabat	RRWWQPRRFSALEHGIYPPQTQSKI	GDLEQEPEPEPEPEVELESELEPQQP .
Microbat	RRPWRPRRFSALEHGIHPPQAQSKI	EDLEQEPEPELEPQPQPQPQP .

A Zinc Clasp Structure Tethers Lck to T Cell Coreceptors CD4 and CD8



LAG3 disrupts CD4:p56^{lck} and CD8:p56^{lck} interaction

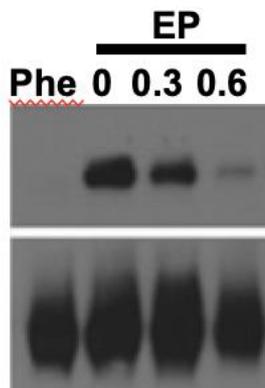
- LAG3 disrupts CD4:p56^{lck} and CD8:p56^{lck} interaction (Co-IP, CDA, PS, STED, STORM)



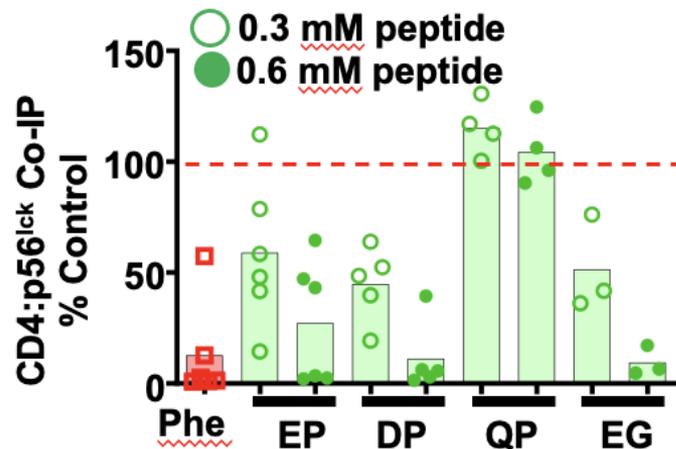
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Motif	Sequence
EP	RELETEMGQEPPEPEPEPQLEPEPRQL
DP	RDLDTDMGQDPDPDPQQLDPDPQRQL
QP	RQLQTQMGQQPQPQPQQLQPQPRQL
EG	RELETEMGQEGEGEGEGQLEGEGRQL



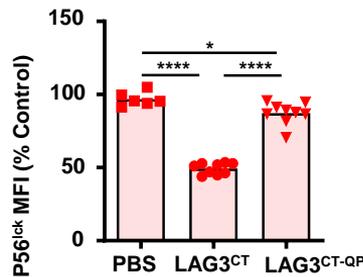
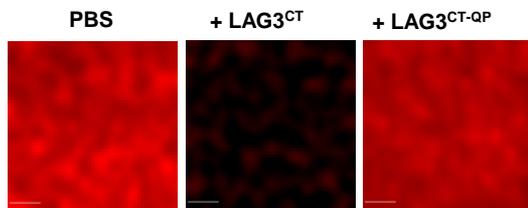
[mM]
 IP: CD4
 IB: p56^{lck}
 Input
 IB: CD4



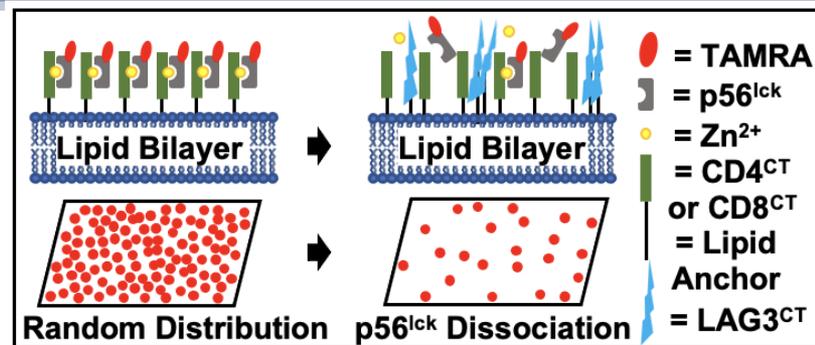
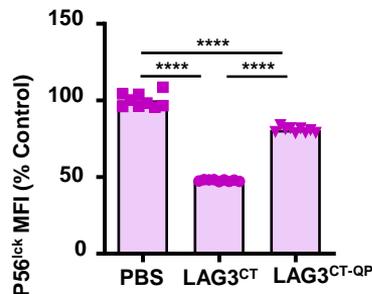
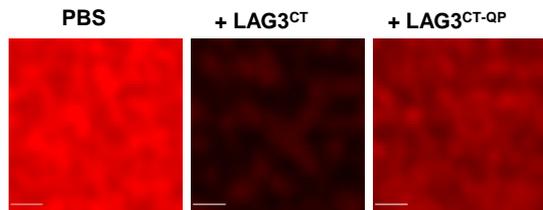
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CD4^{CT} + p56^{lck}

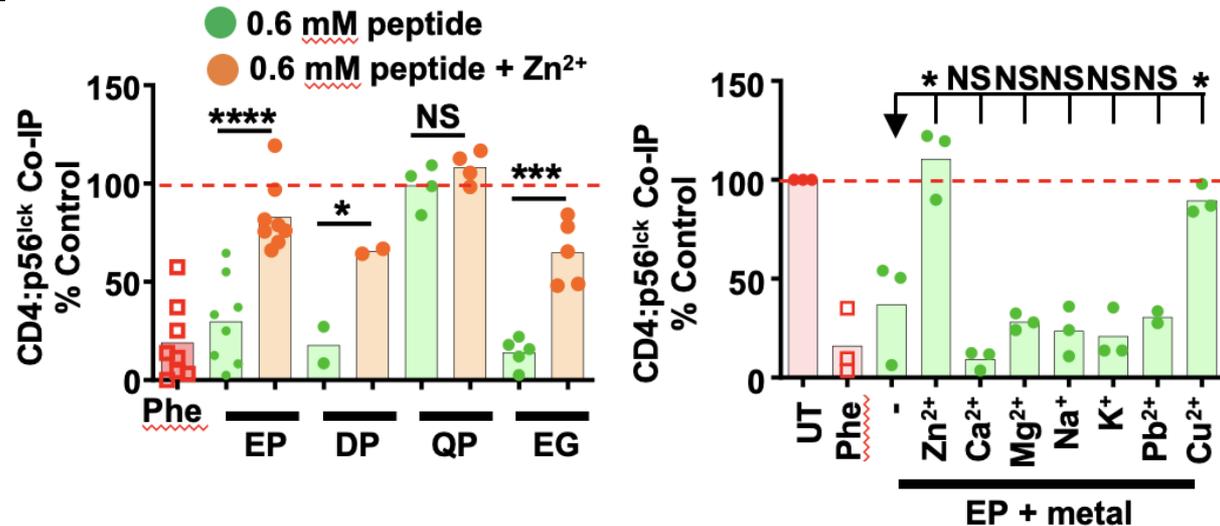
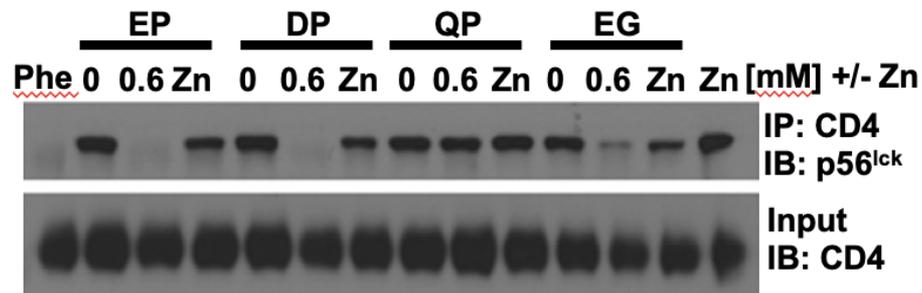


CD8^{CT} + p56^{lck}



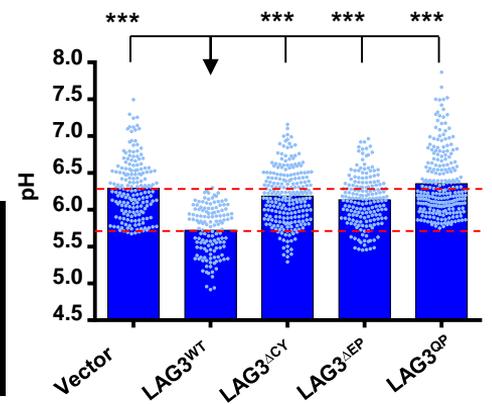
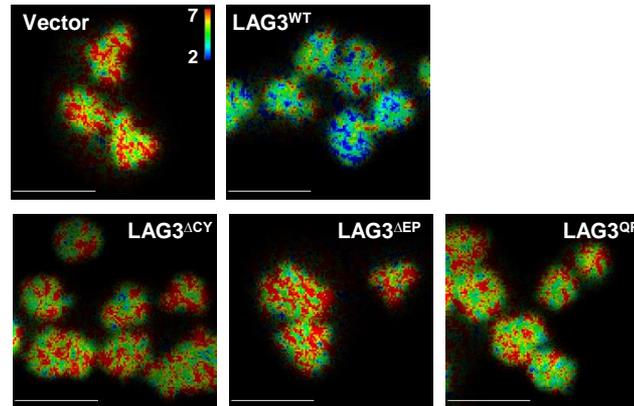
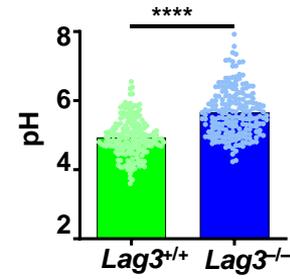
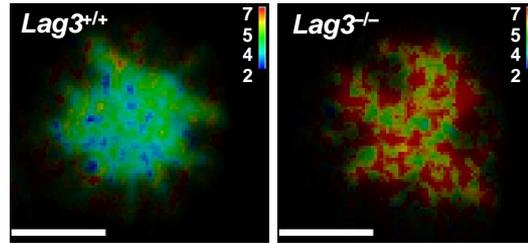
LAG3 'EP' motif disrupts p56^{lck} by binding to Zn²⁺

- LAG3 disrupts CD4:p56^{lck} and CD8:p56^{lck} interaction (Co-IP, CDA, PS, STED, STORM)
- LAG3 'EP' motif disrupts coreceptor:p56^{lck} association by binding to Zn²⁺ (CDA, ITC, NMR)

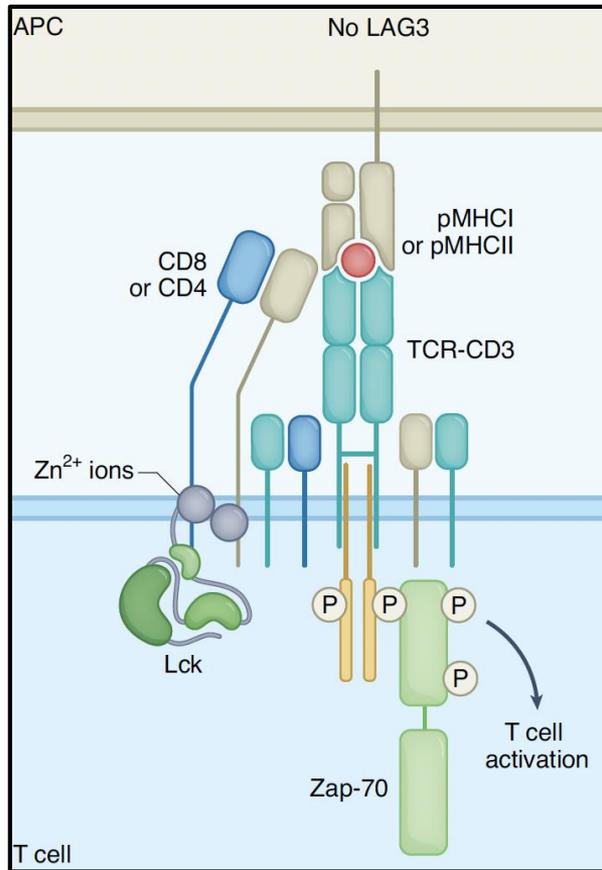


LAG3-EP motif lowers local pH in the IS

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- LAG3 'EP' motif disrupts coreceptor:p56^{lck} association by binding to Zn²⁺ (CDA, ITC, NMR)
- LAG3-EP motif can lower local pH in the IS (FLIM, Confocal)

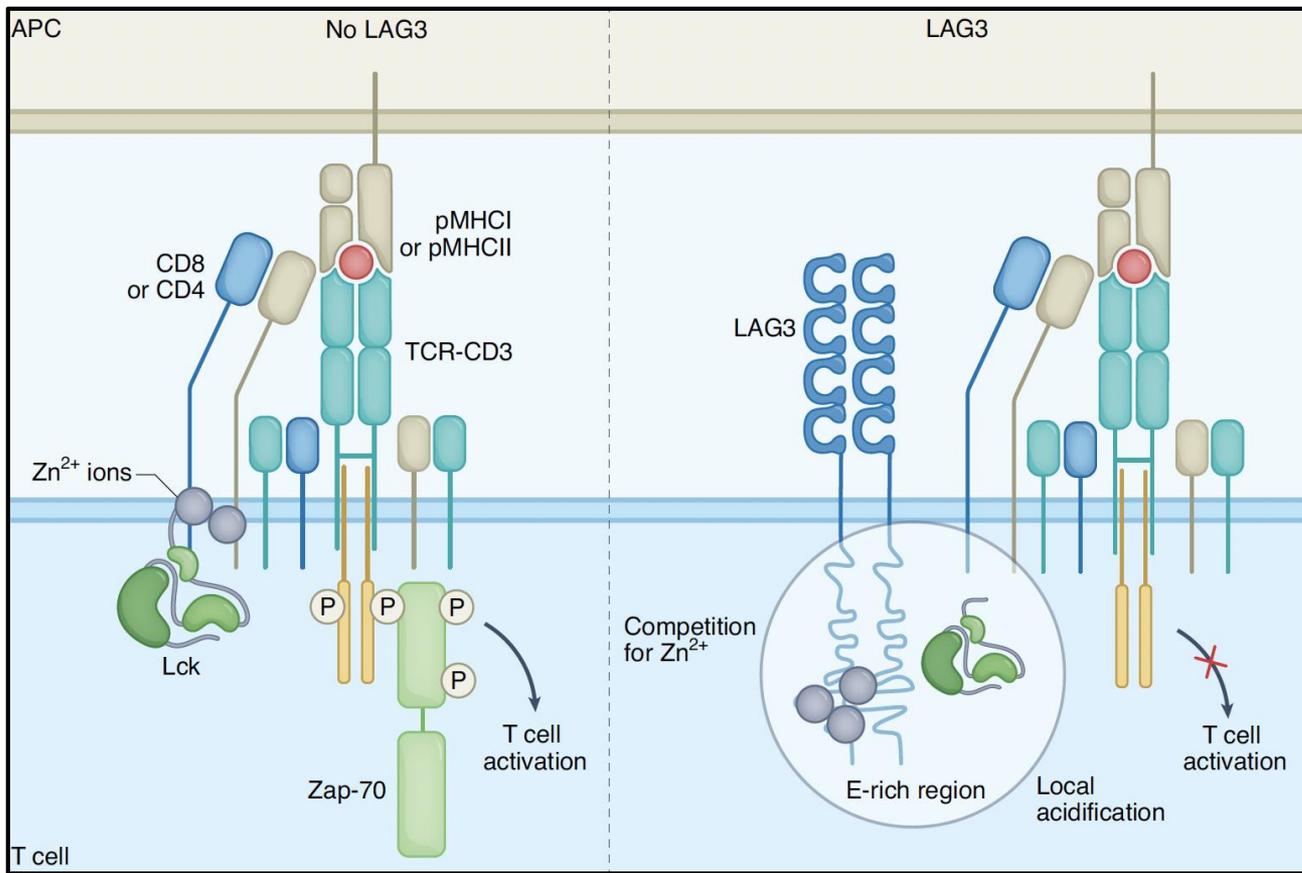


Model for the mechanism of action of LAG3



LAG3 acts as a signal disruptor, and mediates its inhibitory function by associating with the TCR:CD3 complex and inducing co-receptor:p56^{lck} dissociation by reducing the local pH and binding Zn²⁺ in the IS, thereby impacting downstream signaling

Model for the mechanism of action of LAG3



Implications & Future

Questions:

- Implications for ligands?
- Are current LAG3 therapies optimal?
- How does LAG3 work in non-T cells?
- Implications for CAR-T?

Vignali Lab

Past

Maria Bettini

Matt Bettini

Haopeng Wang

Jeff Holst

Lauren Collison

Meghan Turnis

Seng-Ryong Woo

Greg Delgoffe

Andrea Workman

Clifford Guy

Po-Chien Chou

Abby Overacre-Delgoffe

Deepali Sawant

Sherry (Qianxia) Zhang

Hiroshi Yano

Ashwin Somasundaram

Tullia Bruno



Present

Creg Workman

Kate Vignali

Jian Cui

Zhanna Lipatova

Gracie (Chang) Liu

Lawrence Andrews

Tony Cillo

Angela Gocher

Chang Yi

Vaishali Aggarwal

Amayrani Abrego

Anantxa Romero

Chris Chuckran

Sayali Onkar

Stephanie Grebinoski

Feng Shen

Ellen Scott

Erin Brunazzi

Carly Cardello

Maria Raja

Gwen Pieklo

Thank you for listening

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Duyvuri, Seugwon Kim, Ryan J Soose, Dan Zandberg
UPMC Hillman Cancer Center/Dept. Otolaryngology

Jason Luke, John Kirkwood, Hassane Zarour, Bob Edwards, Francesmary Modugno, Ron
Buckanovich, Lan Coffman, Steff Oesterreich, Priscilla McAulliffe, Nduka Amankolor,
Rocky Schoen, Katie Nason, Arjun Pennathur, James Luketich, Laura Stabile, James
Herman, Herb Zeh, Amer Zureikat, Annie Im, Michael Boyidiazzi, Kelly Bailey.
UPMC Hillman Cancer Center

**Patients &
their families**

Marcel Bruchez
CMU

Diana Mitrea, Richard Kriwacki
Jamshid Temirov, Xueyan Liu,
Hui Zhang
St Jude

Simon Watkins,
Flow Cytometry and DLAR
UPitt/Hillman Cancer Center

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