

How does LAG3 work

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UPMC Hillman Cancer Center*

Scientific Director, Fondazione Ri.MED



University of
Pittsburgh

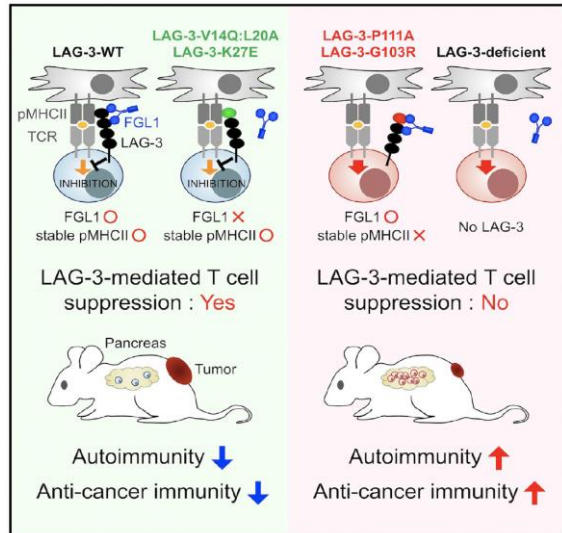
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Disclosures

- 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)

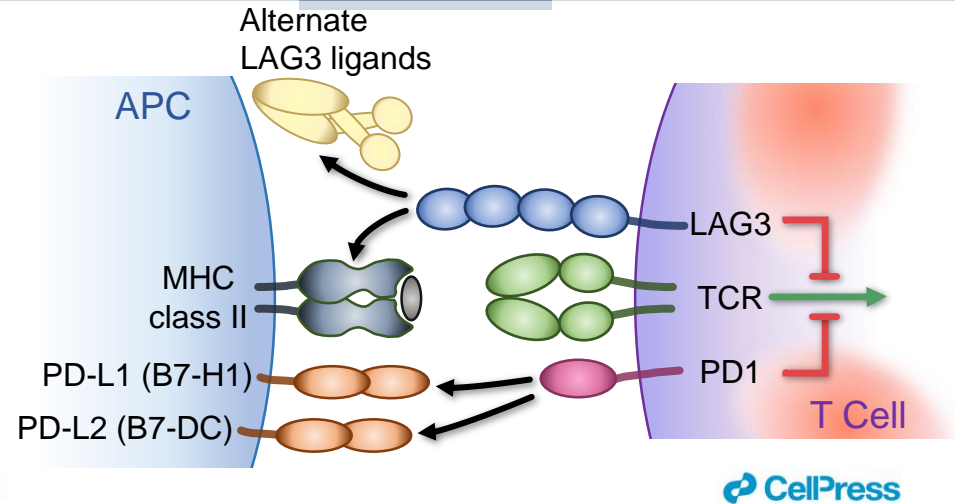


Immunity

Article

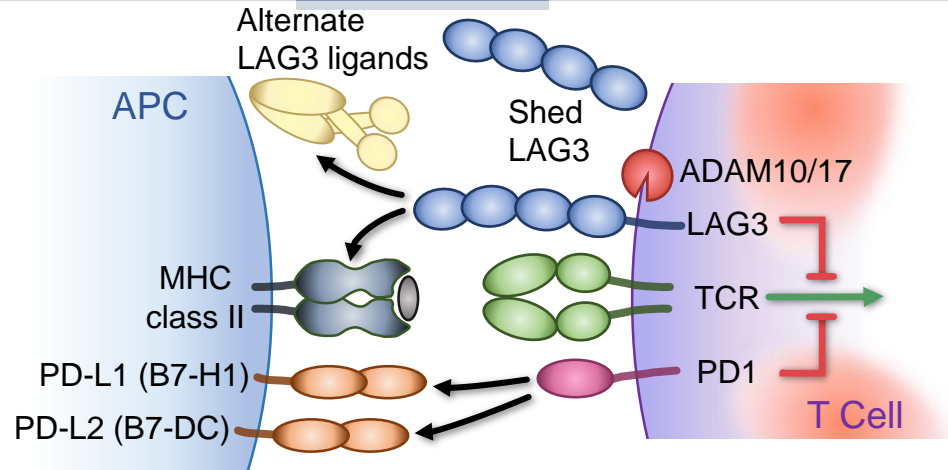
Binding of LAG-3 to stable peptide-MHC class II limits T cell function and suppresses autoimmunity and anti-cancer immunity

Takumi Maruhashi,¹ Daisuke Sugiura,¹ Il-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,*}



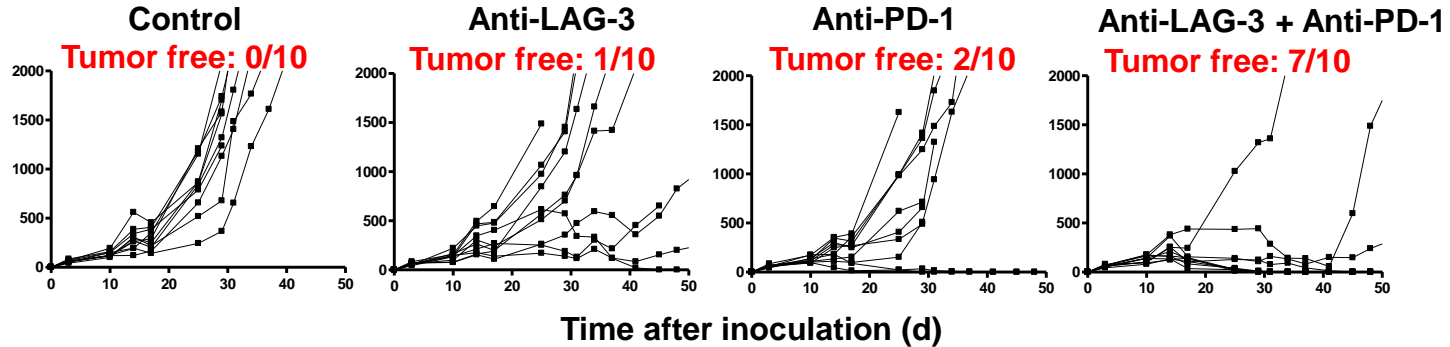
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- LAG3 is rapidly shed by ADAM10/17; high sLAG3 in plasma (*Jl* 2004; *EMBOJ* 2007; *Science Immunology*, 2020)
- Anti-LAG-3 / anti-PD-1 exhibit synergistic combinatorial anti-tumor activity (*CR* 2012)

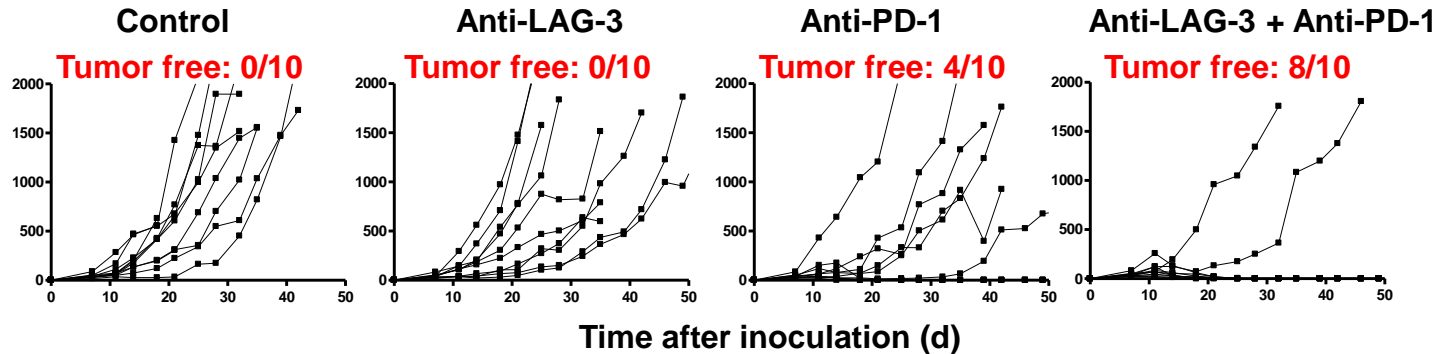


Tumor clearance with combinatorial anti-LAG-3 / anti-PD-1 treatment

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

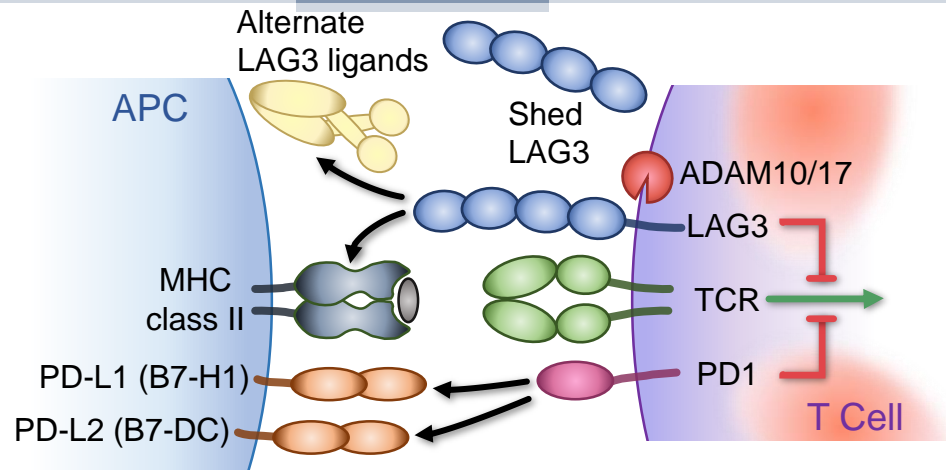


MC38 Adenocarcinoma – C57BL/6 mice (Ab treatment = 10mg/kg at day 8, 11, 14)



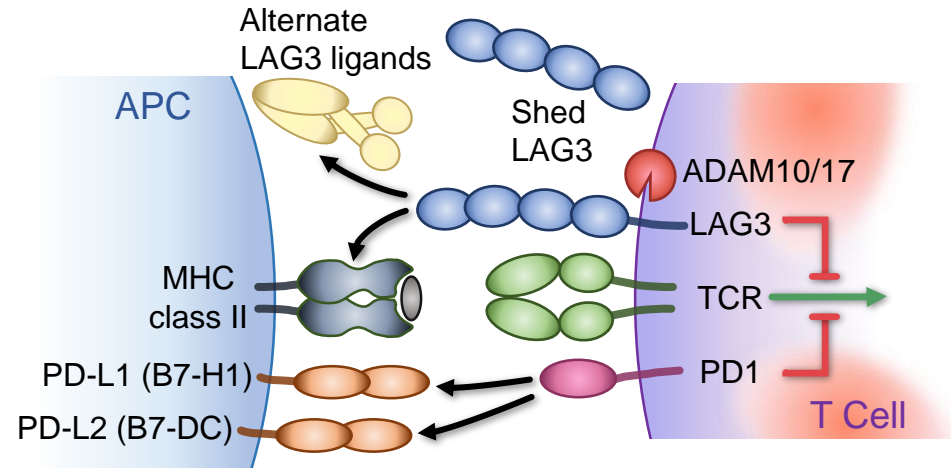
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- Anti-LAG-3 / anti-PD-1 exhibit synergistic combinatorial anti-tumor activity (*CR* 2012)
- Relatlimab (BMS) first-in-clinic: currently over 20 LAG3 targeting therapeutics in clinical trials!
- REALTIVITY-047: Rela + Nivo phase 2/3 trial in treatment-naïve 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



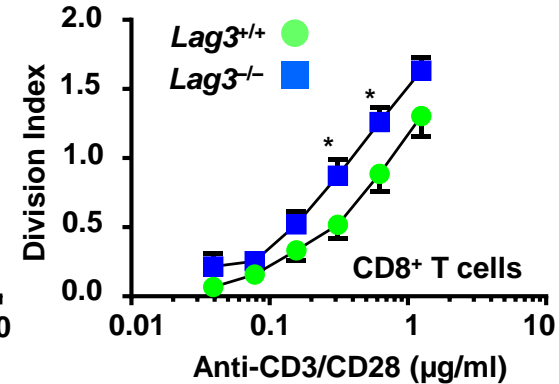
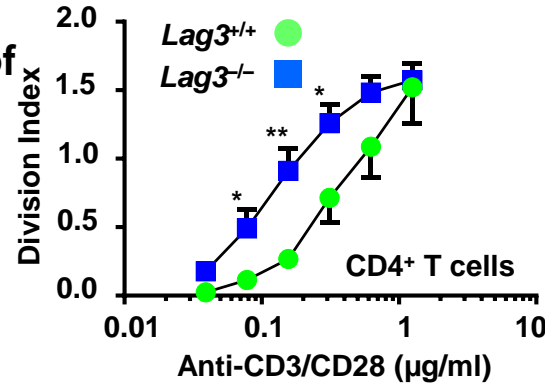
Creg Workman

Curious Observations:

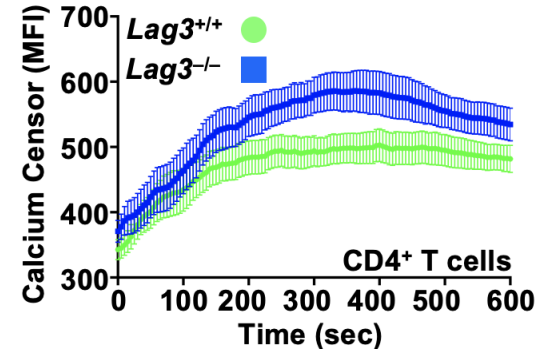
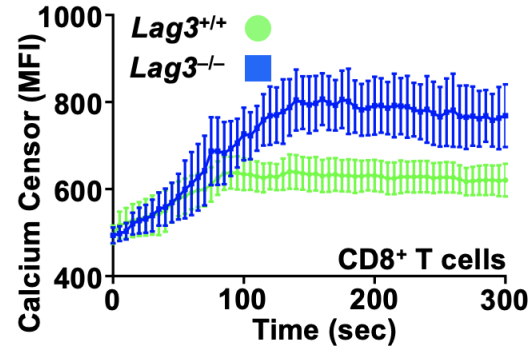
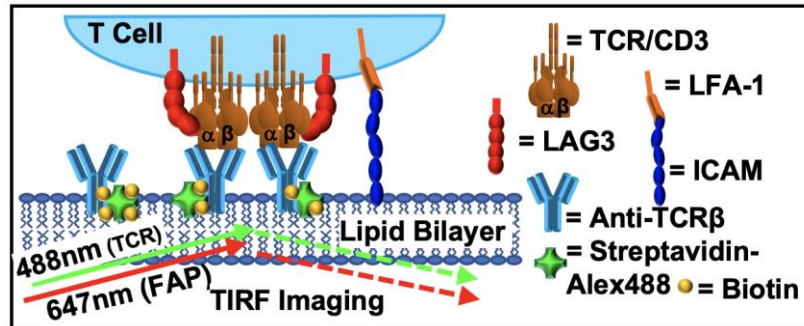
- Our anti-mouse LAG3 blocking mAb (C9B7W) does not block LAG3:MHC class II interaction
- LAG3 inhibitory activity cannot be induced by receptor crosslinking
- LAG3 function is co-receptor (CD4/CD8) dependent (*J I* 169:5392, 2002)

LAG3 can function in the absence of MHC class II

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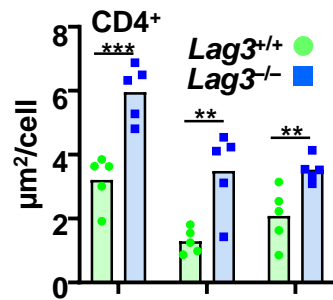
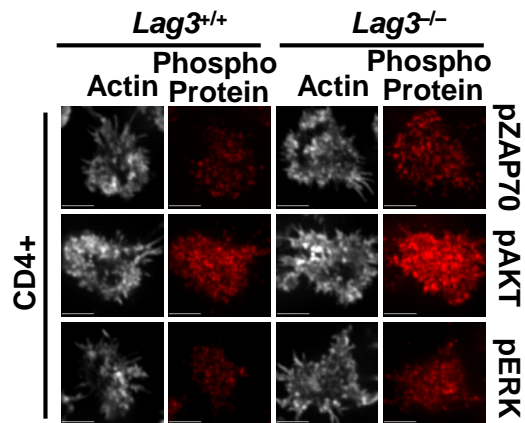
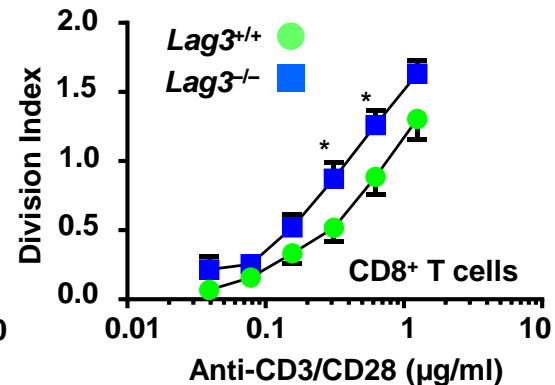
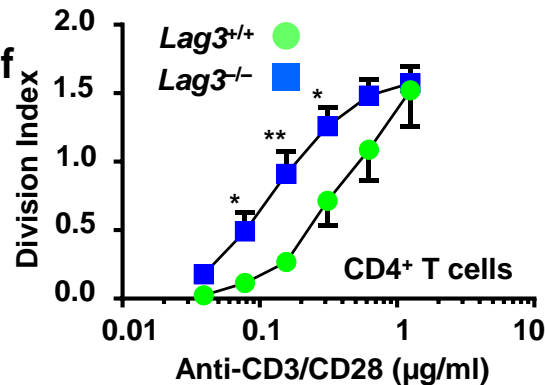


Total Internal Reflection Fluorescence (TIRF) Microscopy



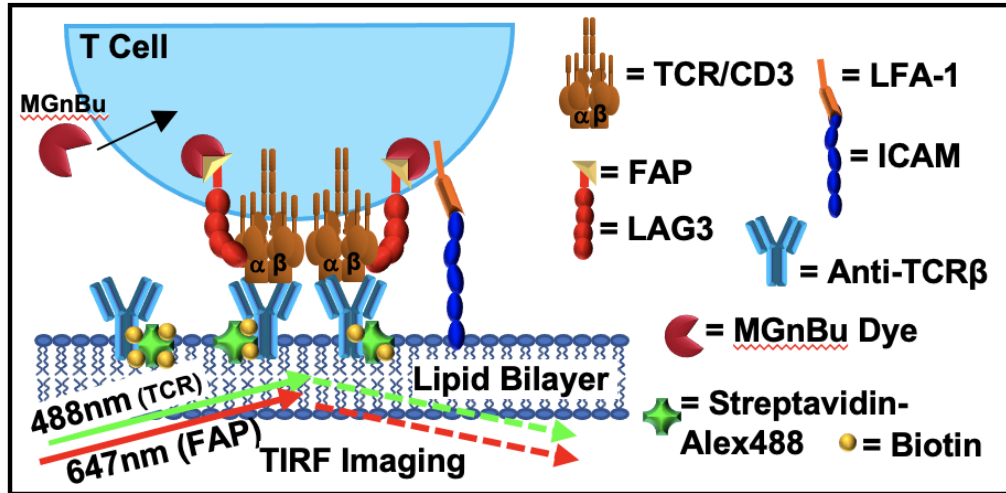
LAG3 can function in the absence of MHC class II

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LAG3 associates with TCR/CD3 complex

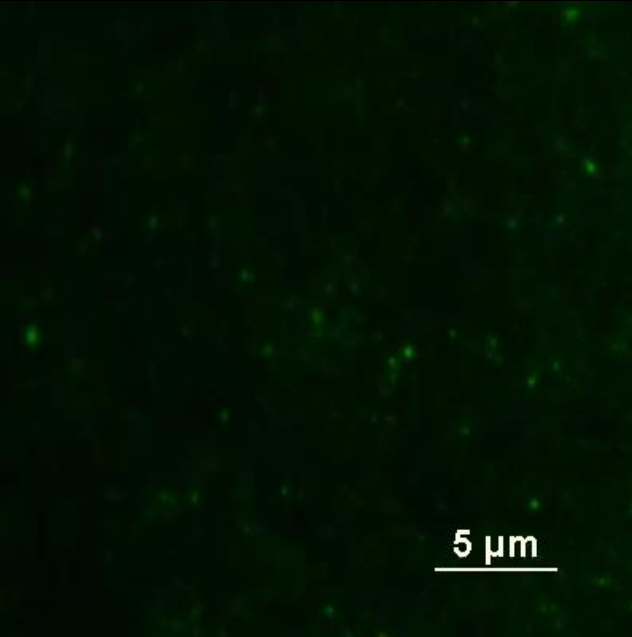
- LAG3 can function in the absence of MHC class II
- LAG3 associates with TCR/CD3 complex:
~13:1 (TIRF, STED, STORM, Expansion Microscopy, Co-IP) [**ligand in cis**]



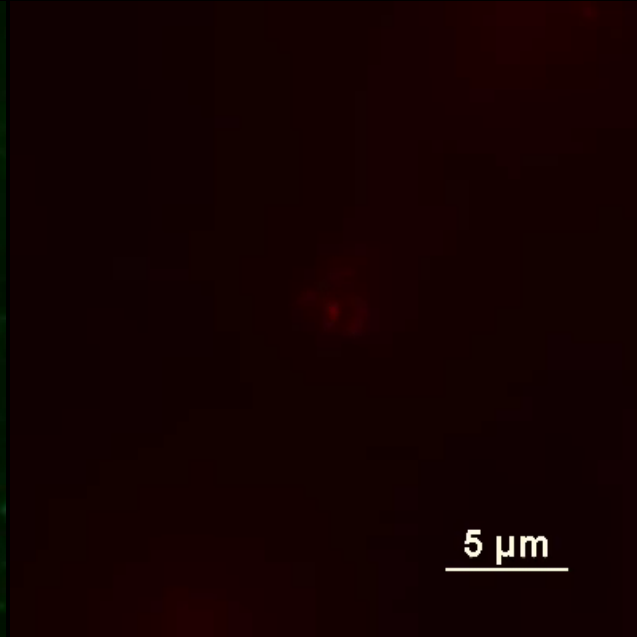
Total Internal
Reflection
Fluorescence
(TIRF) Microscopy

LAG3 tracks with the TCR into the IS

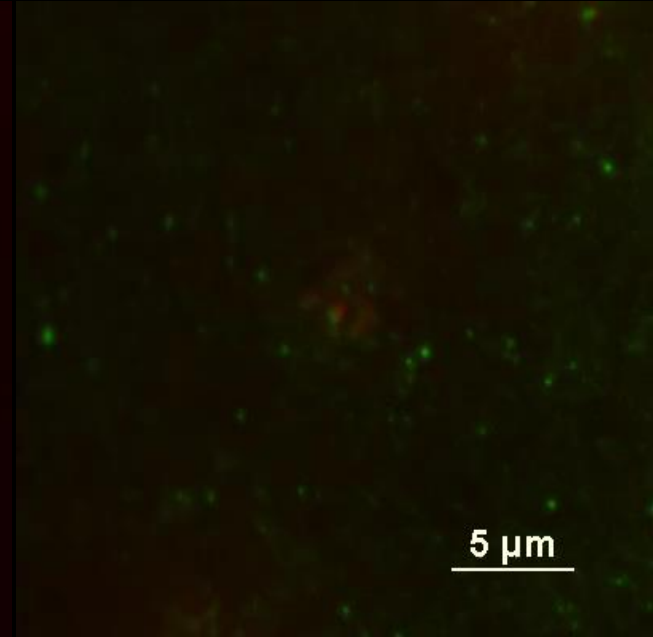
TCR



LAG3

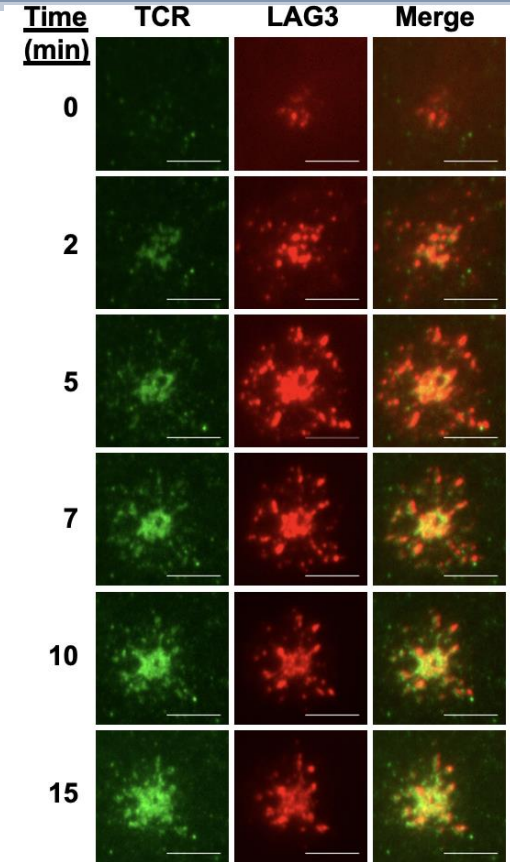
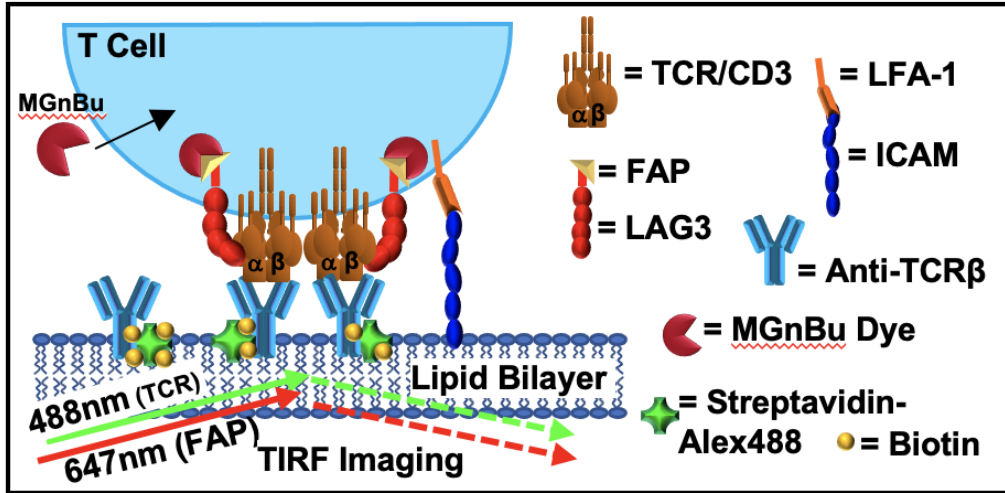


TCR + LAG3



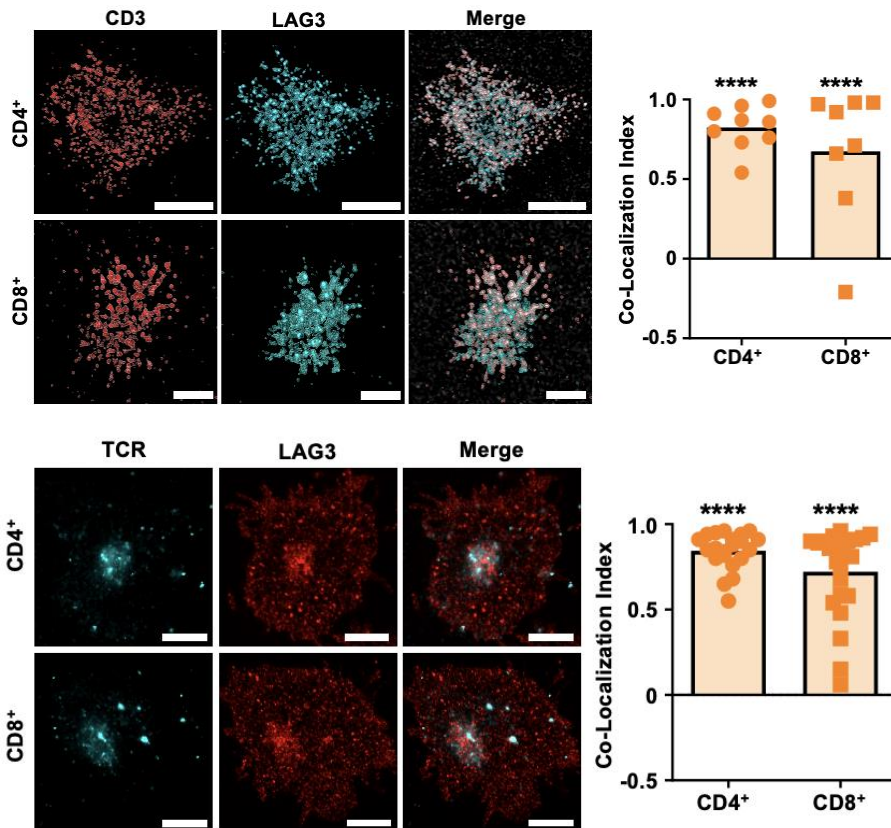
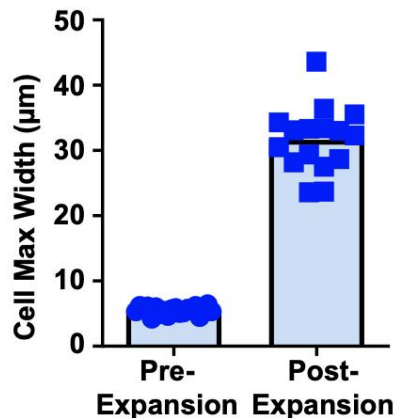
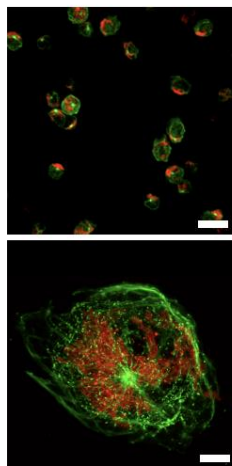
LAG3 associates with TCR/CD3 complex

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



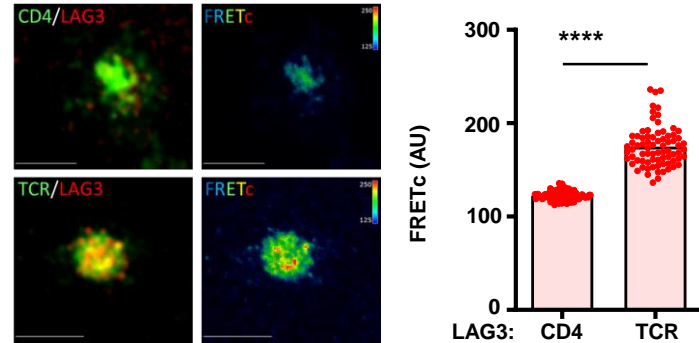
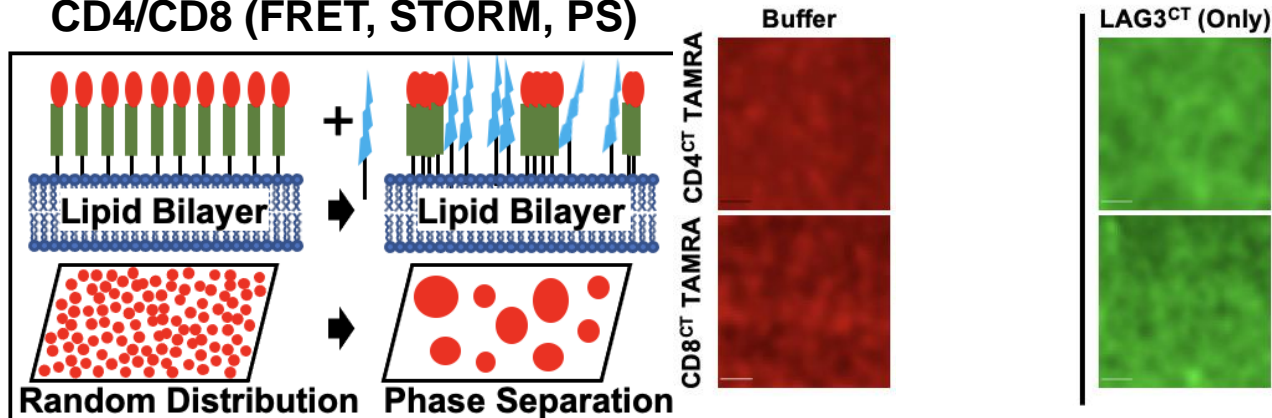
LAG3 associates with TCR/CD3 complex

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LAG3 also closely associates with CD4/CD8

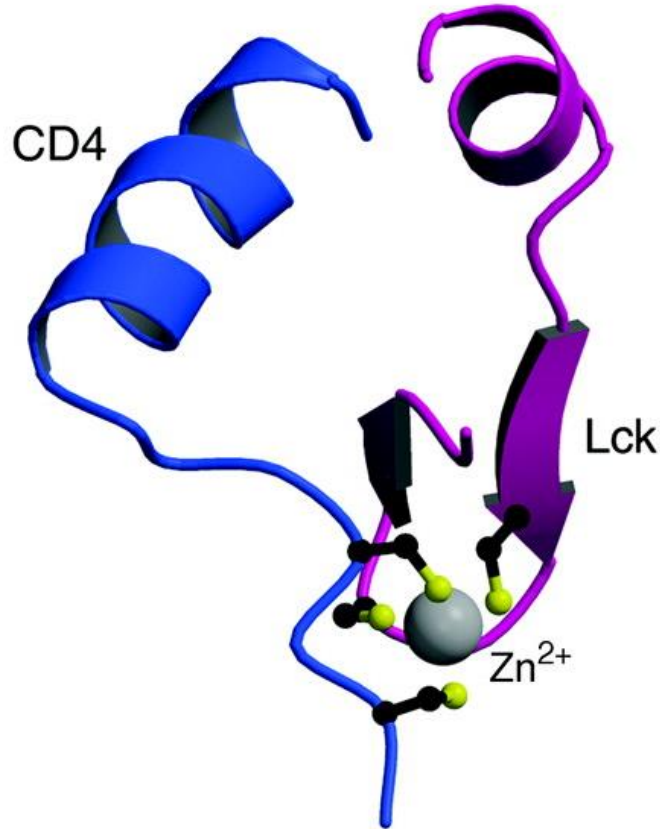
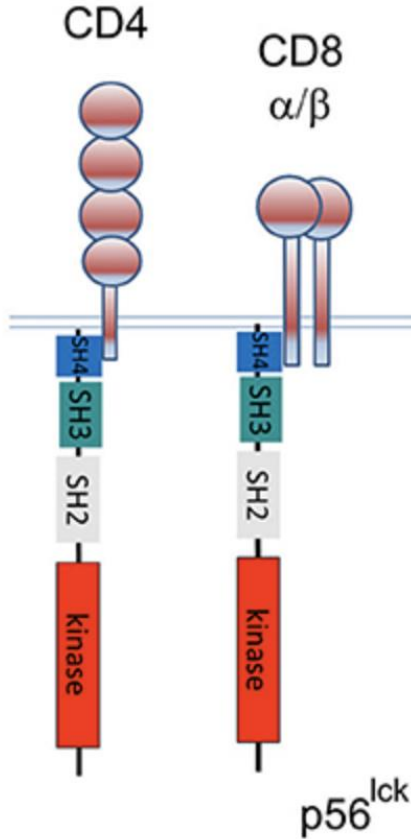
- LAG3 can function in the absence of MHC class II
- LAG3 associates with TCR/CD3 complex: ~13:1 (TIRF, STED, STORM, Expansion Microscopy, Co-IP) [ligand in cis]
- LAG3 also closely associates with CD4/CD8 (FRET, STORM, PS)



Phylogenetically conserved LAG3 repetitive 'EP' motif

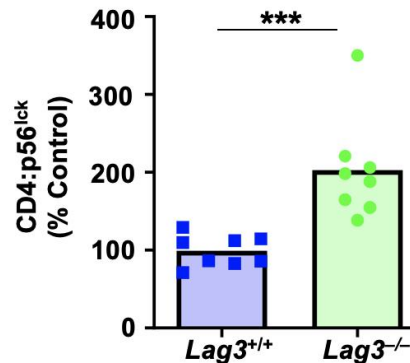
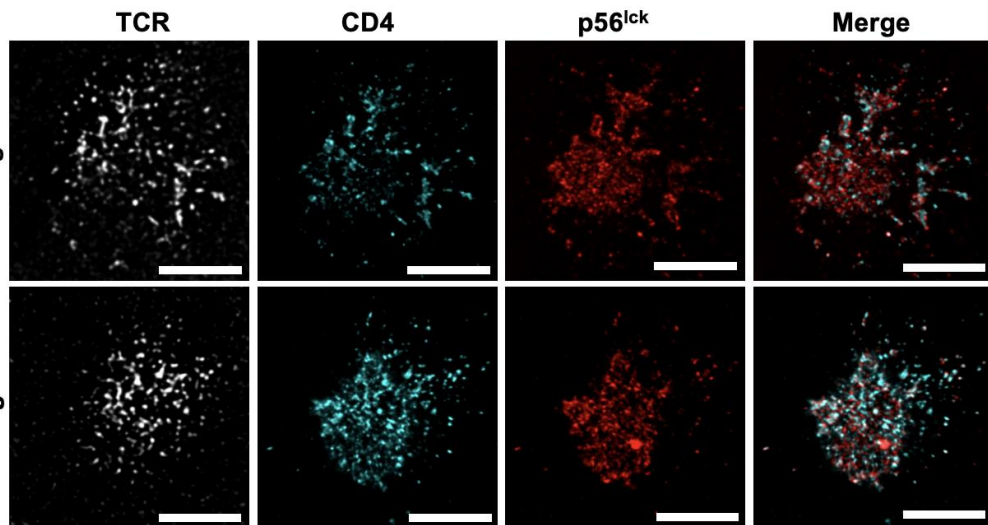
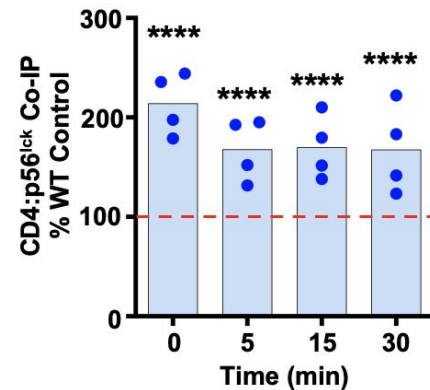
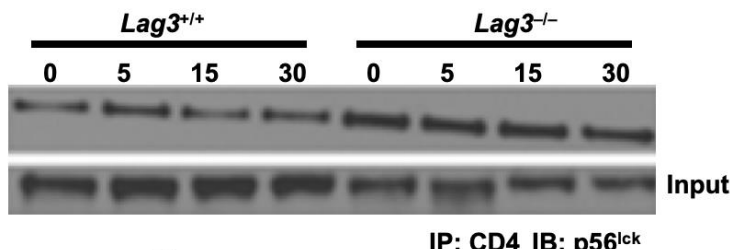
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Rat	RRQLLRRRFSAL E HGIRPPPVQSK I EELEREPE TEME PEPE TEPD PEPQPEPE LE PE SRQL
Human	RRQWRPRRFSAL E QGIHPPQAQSK I EELEQE PEPEPEPEPEPEPEPEPEPEQL.
Gorilla	RRQWRPRRFSAL E QGIHPPQAQSK I EELEQE PEPEPEPEPEPEPEPEPEPEQL.
Chimpanzee	FSQWRPRRFSAL E QGIHPPQAQSK I EELEQE PELEPEPEPEPELGP PEPEPE QL
Orangutan	RRQWRPRRFSAL E QGIHPPQAQSK I EELEQE PELEPEPEPEPEPEPE PQPEPE QL
Gibbon	RRQWRPRRFSAL E QGIHPPQAQSK I EELEQE PEPEPEPEPEPEPEPELGP PEPKPE QL
Macaque	RRQWRPRRFSAL E QGIHPPQAQSK I EELEQE PELEPEPEPELE RE LGPEPEPG PEPEPE QL
Marmoset	RRQWRPRRFSAL E QGIHPPQAQSK I EELEQE LEPEPEPEPELEPEPEPE PERAPE PGPEQL
Bushbaby	KRPWRPRRFSAL E HGIHSPQAESK I EGD QDE PDLEPEPELD PEIGPE LEPGLD PELEPE LAL E QL
Mouse Lemur	RRPWRPRRFSAL E DGIHPPHAESK I EGLE QE LEPEPELE QEPE LGLE LE QL
Panda	RRQWRPRRFSAL E HGTHPPQAQSK I GELE QE PELEPEPEPELELEVE PESE LEPELEPEPEPE
Elephant	RRPWRPRRFSAL E NGIHPPQAQSK I EELELEPEQE MEPEPELELELESE PE
Horse	RRQWRARRFSAL E HGIHPPQAQSK I EELEPE AQP ETELALE PD PELELE QP
Cow	RRQW-PRRFSAL E HGTHPSQASSK I GELEPELEPEPD PEVEPEPEPEPE ESQPQL QPEQ P
Pig	RRRWPRRFSAL E HGTHPPQAQSK I GELEPEPELEPEPEPELEVE PEPQPE QP
Dog	GLKWRPRRFSAL E LGHPPQAQSK I GELE QE PELELEPEPEPELEPEPEPEEL
Cat	RRQWRPRRFSAL EHE IHPPQTQSK I GELEPEPELEPEPEPEPEPEPEPEQL
Guinea Pig	KRQWRSRRFSAL E FGIRPPQAQSK I EEVEQE ADLE TE TPQSCSLGPQQPPSPFFHHCAGC
Kangaroo Rat	RRQWRPRRFSAL E LGTYPQAQSK I EEWELDM EP EME QELEPPTE PE LTQL
Pika	RRQWRPRRFSAL E HGAPPPHAQSK I EELEPEEL Q PEPEPE PELGLE PE PRQL
Rabbit	RRQWRPRRFSAL E HGAPPPQAQSK I AASSVSPSPSP EE SLLPGCVKPSPLPSAALPPTGCQL
Squirrel	RRQWRPRRFSAL E HGIHPPQSQSK I EEPEQE PEPEPEPEPEPE QEPEPE LELL
Shrew	RRQWRPRRFSAL E QGVHPPEAQGK I EELEQD PELEPGTE PEPEPE PELEPA PELE QSR
Tree Shrew	RRRWPRRFSAL E HGIDPPQAQGK I EELEQGLE LEPEPEPG PEPGPE PEHF.
Wallaby	RPIQLPRRFSAL E CAAQSSHGQNK I EEMERE PVSGLEPHQ EL KMGQL.
Tasmanian Devil	RQGQFLRSFSAL ED AAQN PQR QSK I EEMEPE CPCQS.
Megabat	RRWWQPRRFSAL E HGIYPPQTQSK I GDLE QE PEPEPEPEVE LESE LEPQQP.
Microbat	RRPWRPRRFSAL E HGIHPPQAQSK I EDLEQE PEPELE PEPQPQPQPQP QP.

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



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

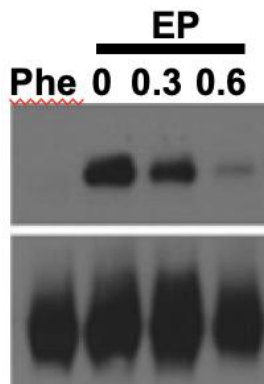
- LAG3 disrupts CD4:p56^{lck} and CD8:p56^{lck} interaction (Co-IP, CDA, PS, STED, STORM)



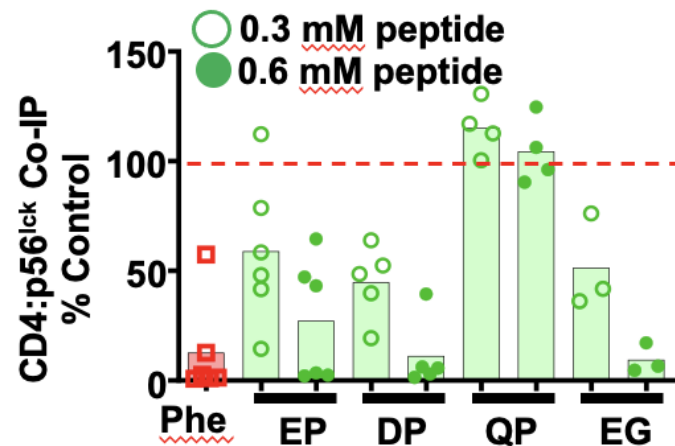
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Motif	Sequence
EP	RELETEMGQEPEPEPEPQLEPEPRQL
DP	RDLDTDMGQDPPDPDPQLDPPRQL
QP	RQLQTQMGQQPQPQPQLQPQPRQL
EG	RELETEMGQEGEGEGEGQLEGEGRQL



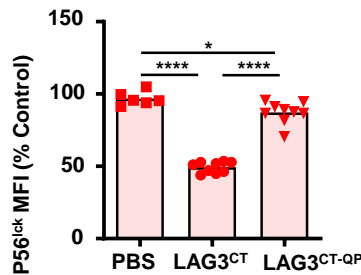
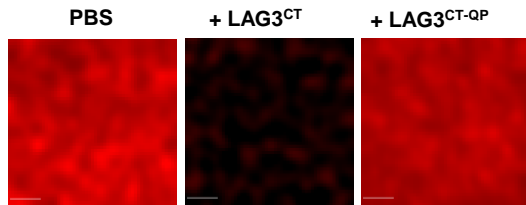
[mM]
 IP: CD4
 IB: p56^{lck}
 Input
 IB: CD4



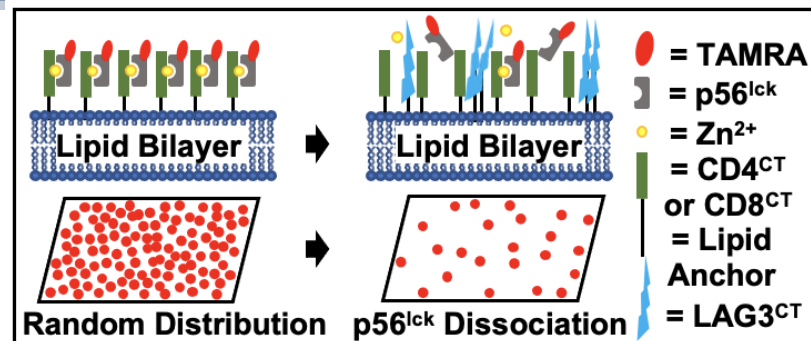
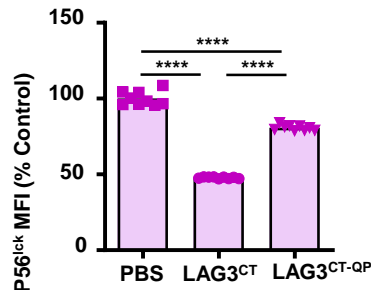
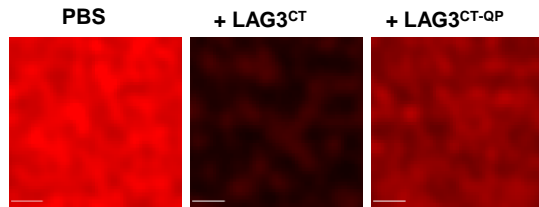
LAG3 disrupts CD4:p56^{lck} and CD8:p56^{lck} interaction

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CD4^{CT} + p56^{lck}

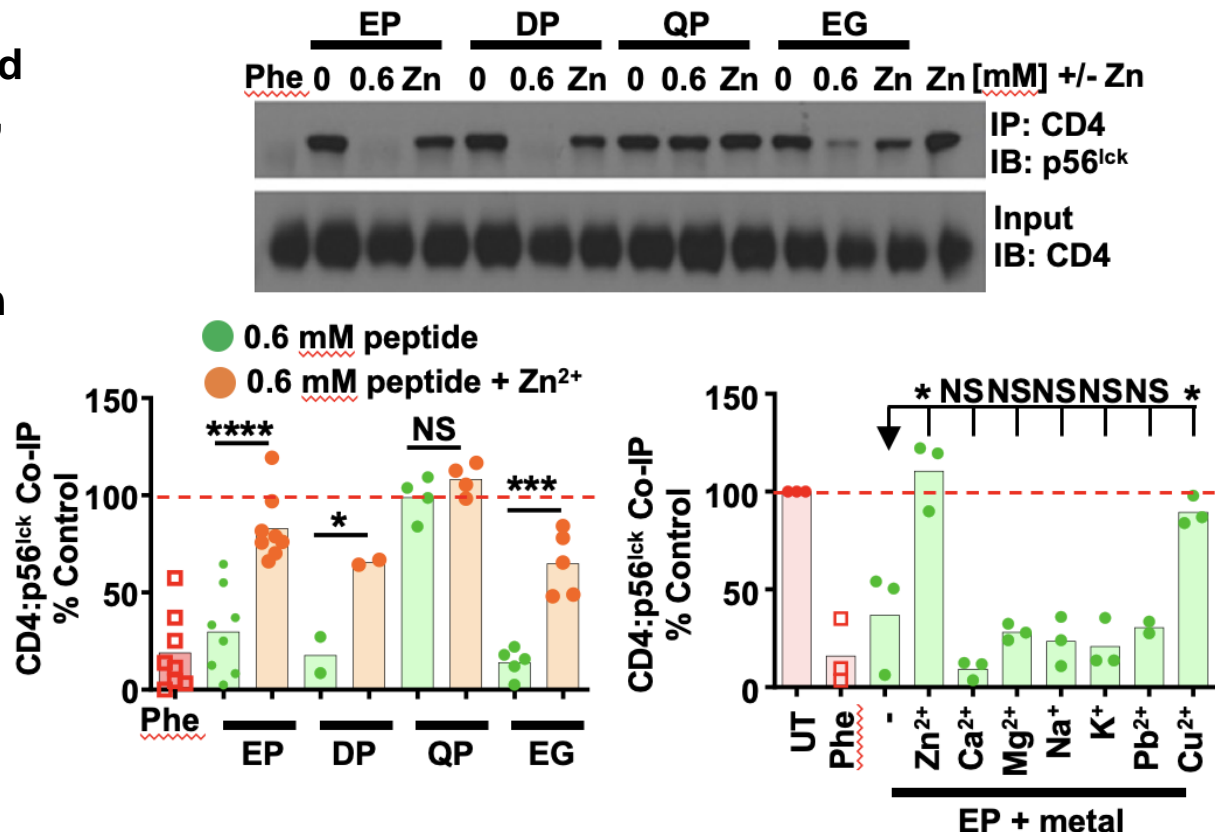


CD8^{CT} + p56^{lck}



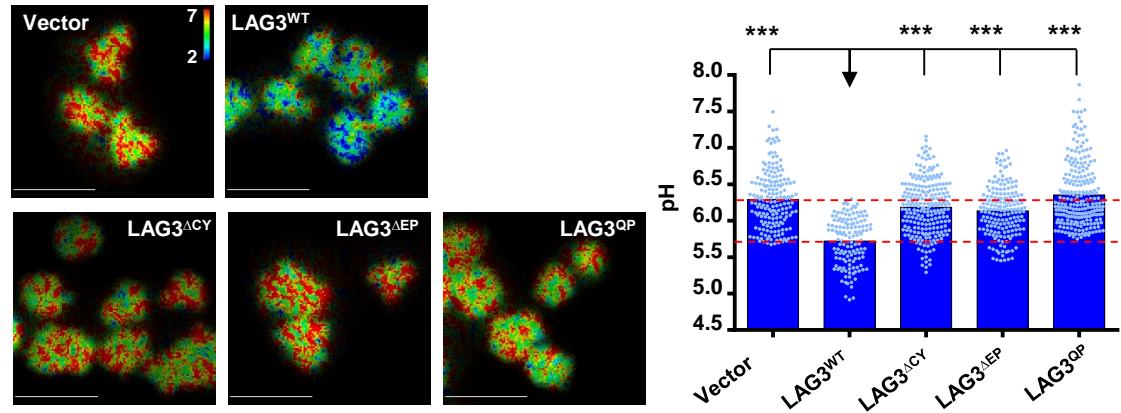
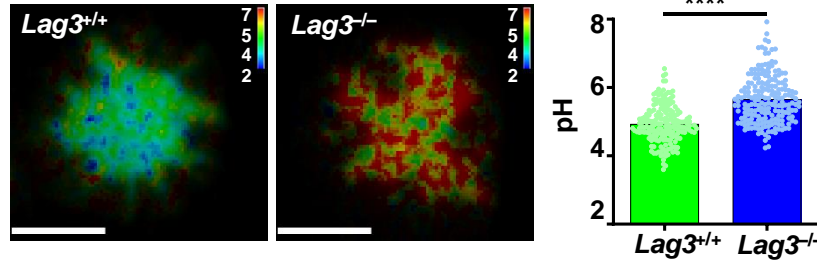
LAG3 'EP' motif disrupts p56^{lck} by binding to Zn²⁺

- 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)

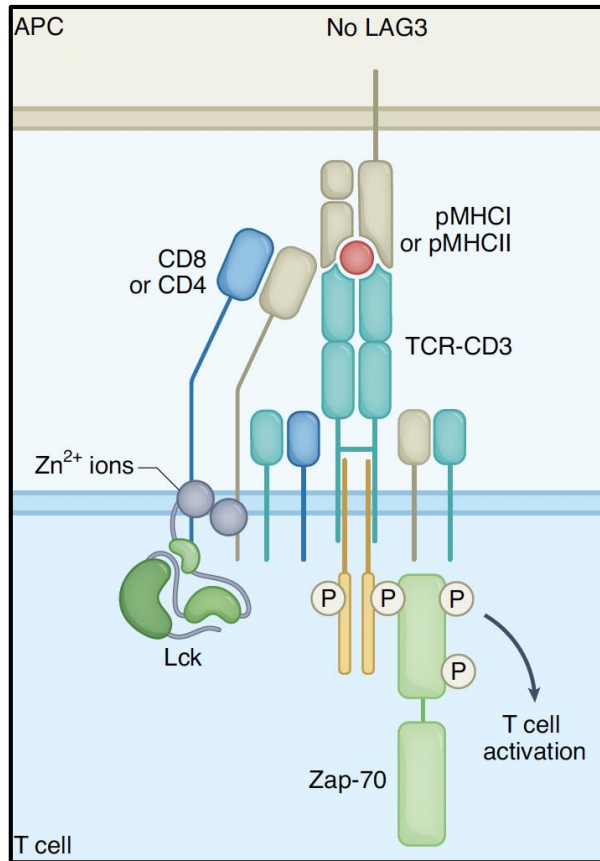


LAG3-EP motif lowers local pH in the IS

- 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 can lower local pH in the IS (FLIM, Confocal)

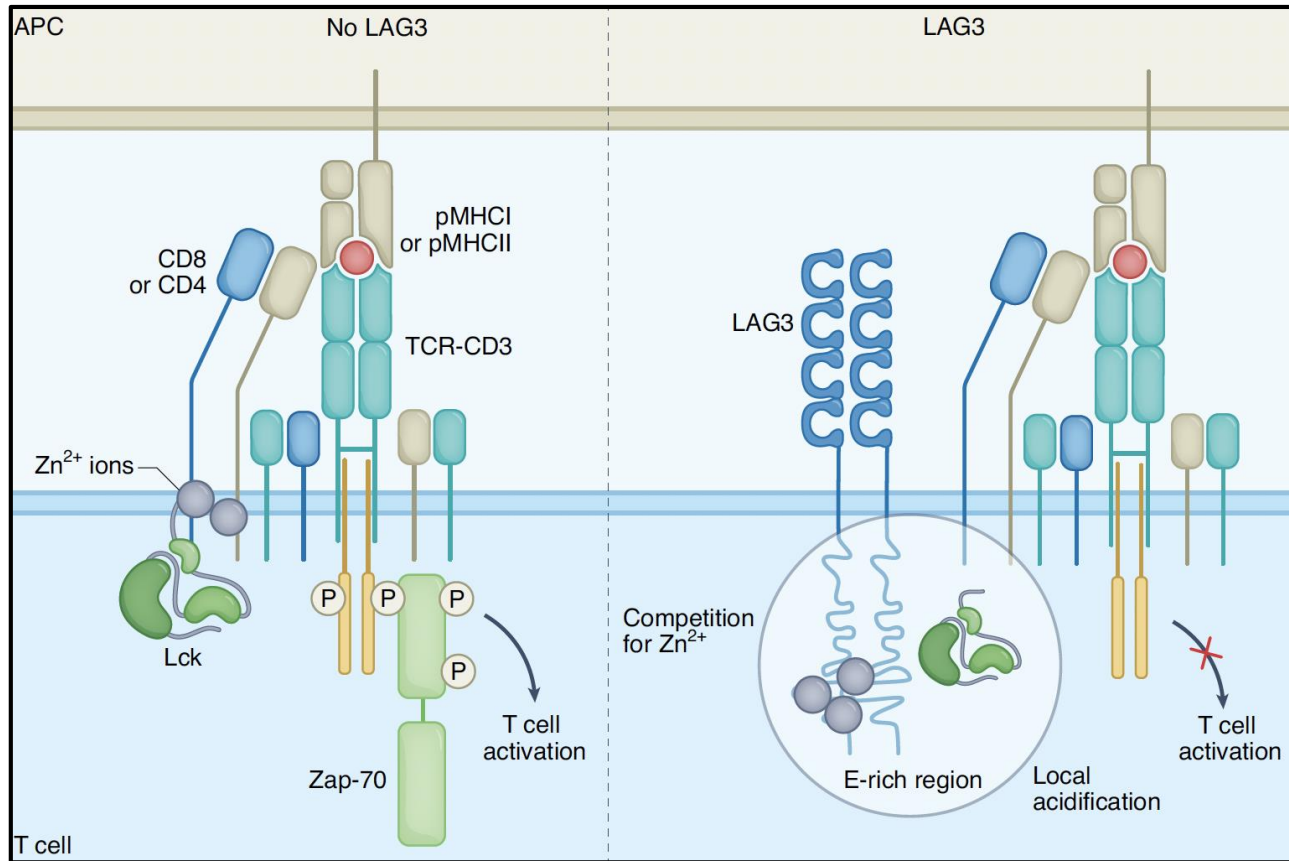


Model for the mechanism of action of LAG3



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, Steffi Oesterreich, Priscilla McAulliffe, Nduka Amankulor,
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

NIH: R35 CA263850; P01 AI108545; R01s DK089125, AI129893 & AI144422; P50 SPORE CA097190 & CA254865