Linh Tran – Goodrum Lab

04.14.2020

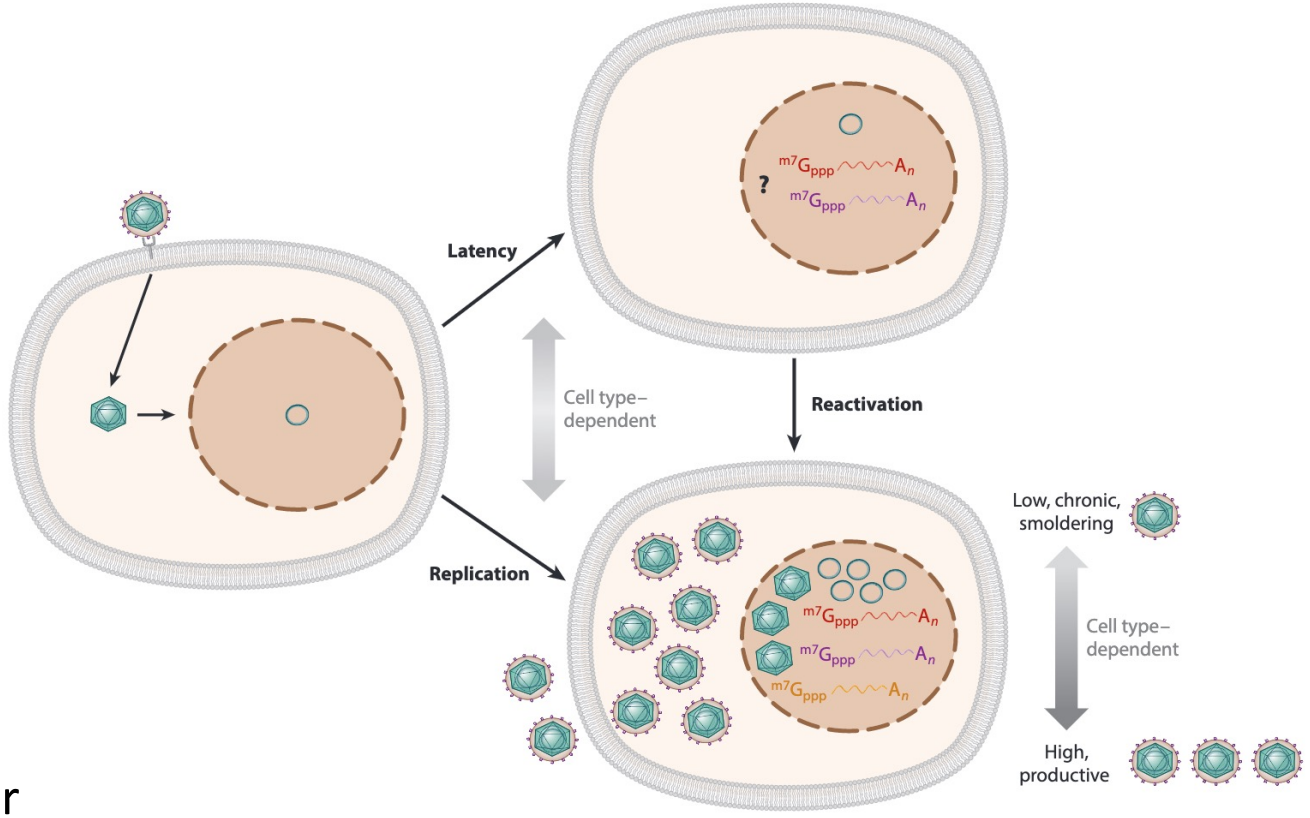
Human cytomegalovirus protein UL135 mediates myelosuppression of hematopoietic progenitor cells

Human Cytomegalovirus (HCMV) Clinical Relevance

- One of nine human herpesviruses (HHV5)
- Host-specific (HCMV, MCMV, RhCMV, etc.)
- Establish **life-long infection** in host
- **Asymptomatically persists** in 40–100% of the world's population
- Complications: immunocompromised/

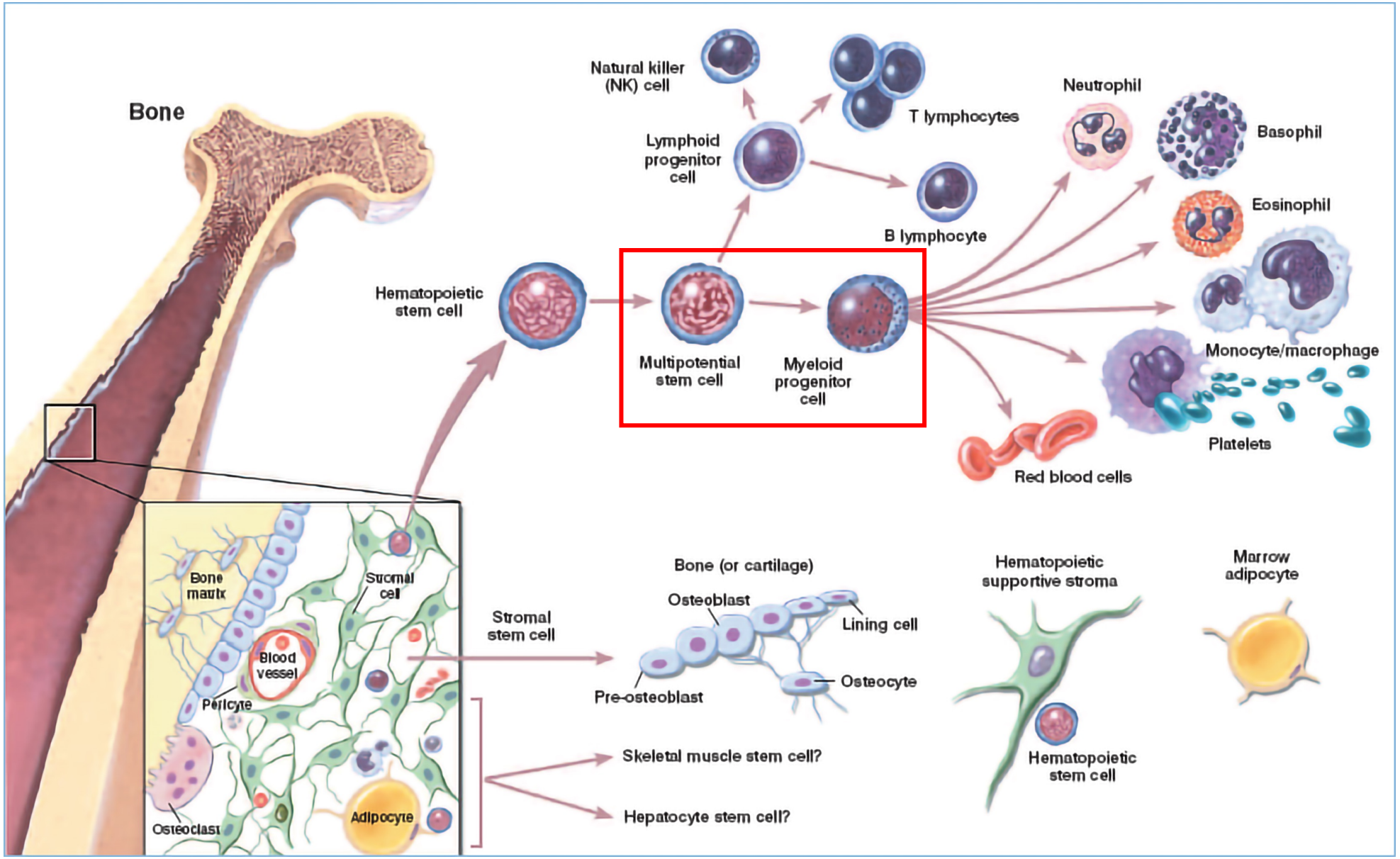
suppressed individuals (congenital CMV, AIDS patients, **bone marrow transplant**, etc.)

- Broad tropism for cell types: **different infection states in different cell types**
- β-herpesvirus:
  - **Latent reservoir** in hematopoietic progenitor cells (HPCs) in the bone marrow and myeloid cells



Goodrum, *Annual review of virology* 3 (2016)

Human Hematopoiesis & HCMV infection implications



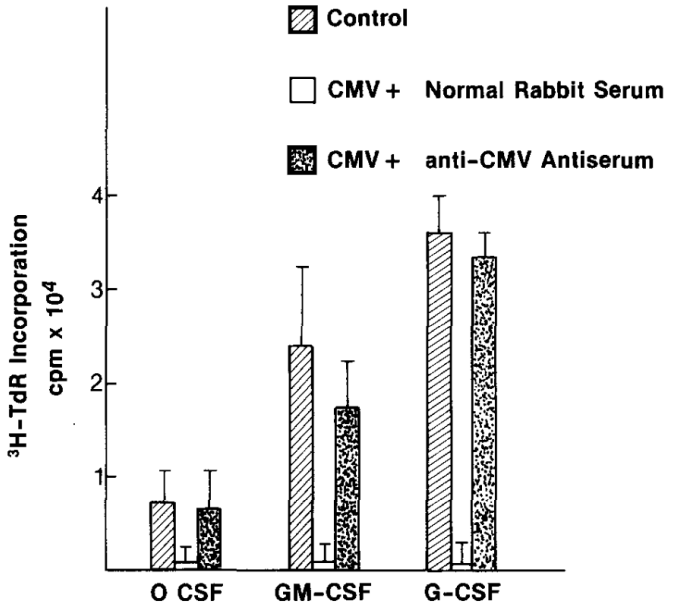
HCMV is the most common infectious cause of morbidity after bone marrow transplantation (BMT)

Table II. Prospective HCMV study 1981-1985

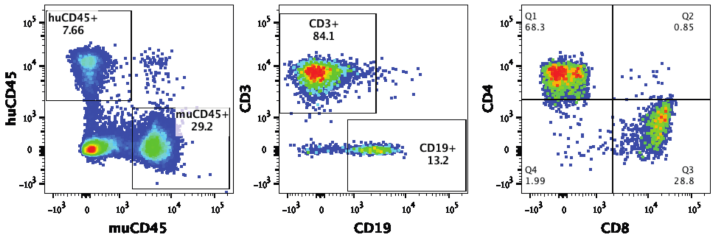
Total patients[a]	127	(%)
Sporadic viremia[b]	20	(15.7)
Persistent viremia[c]	48	(37.7)
No viremia	59	(46.4)

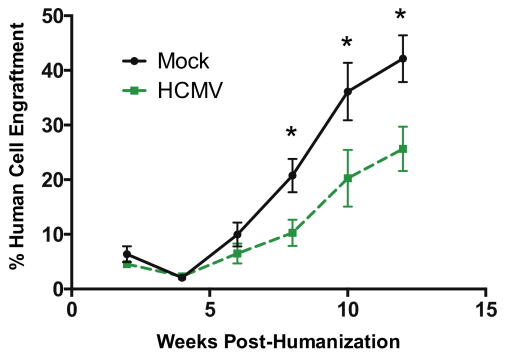
[a]All patients with ≥ 5 blood cultures for HCMV between day 28-day 105 after BMT.
[b]Patients with 1 or 2 positive HCMV blood cultures.
[c]Patients with ≥ 3 positive HCMV blood cultures.

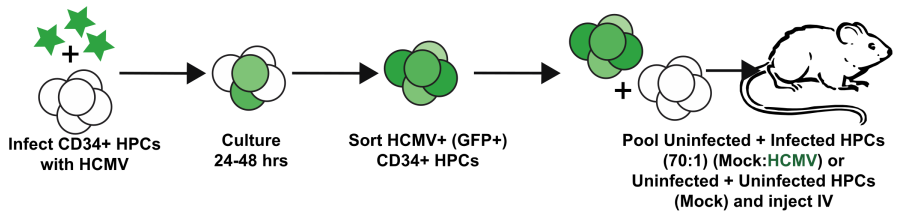
Zaia JA, Int J Cell Cloning (1986)

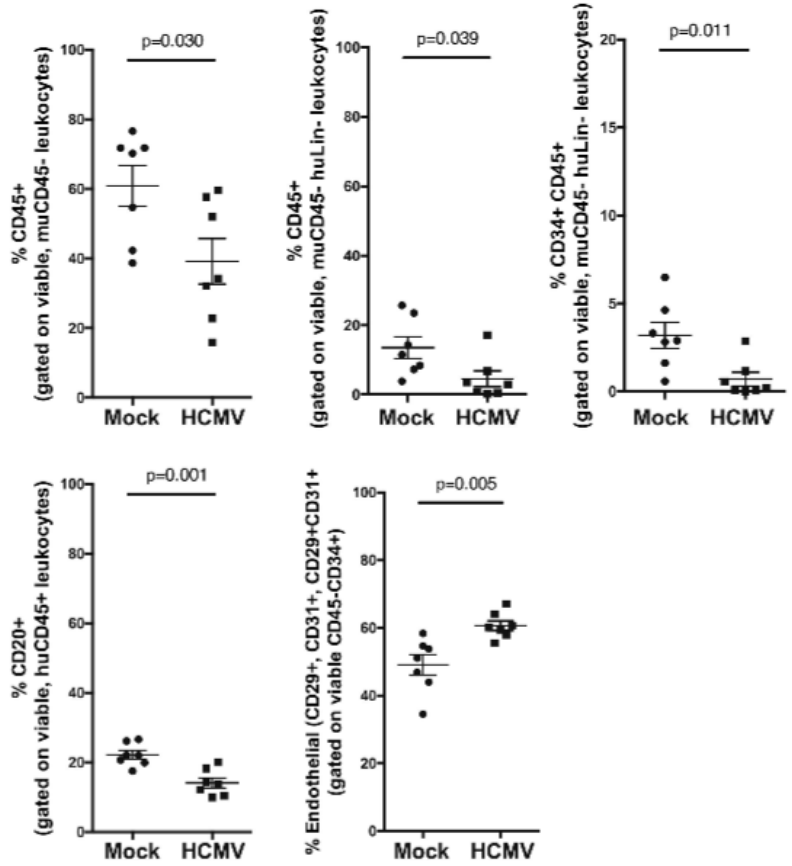


Sing and Ruscetti, Blood (1990)









Crawford et al, Microorganisms (2020)

Long-term goal

Identify the molecular mechanisms of HCMV-induced myelosuppression in hematopoietic progenitor cells (HPCs)

Colony Forming Unit (CFU) Assay

- Each individual progenitor cell is a colony-forming unit.
- Measure ability of each CFU to proliferate and differentiate (hematopoietic potential)
- Frequency and types of progenitor cells present in the original population

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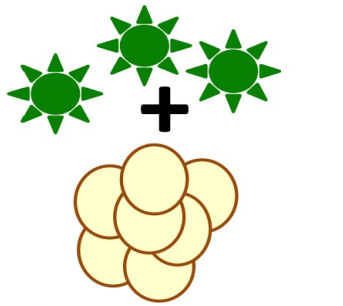

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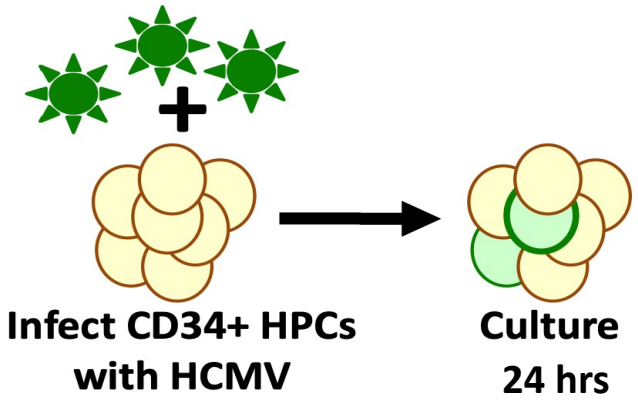

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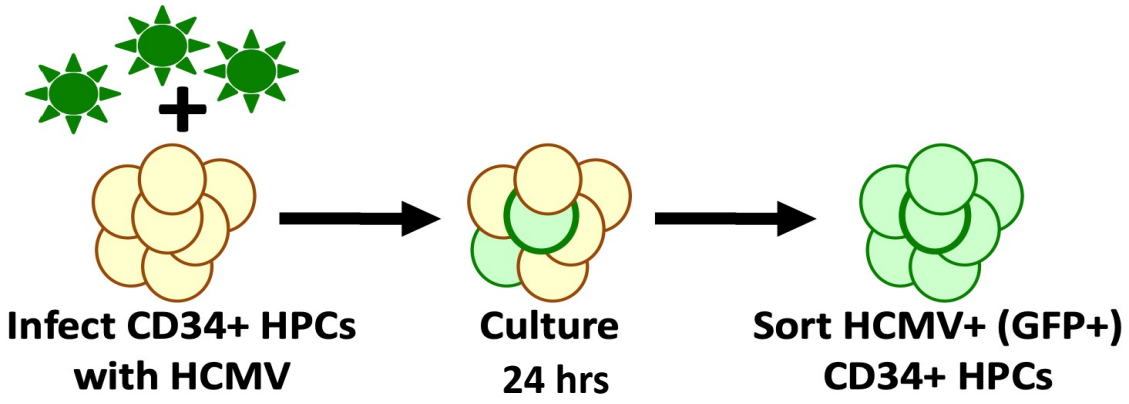

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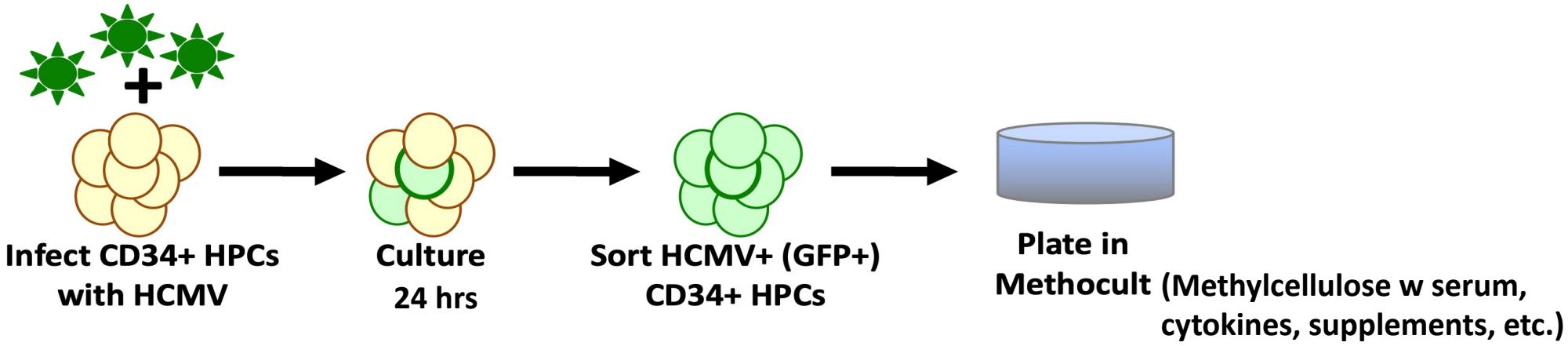

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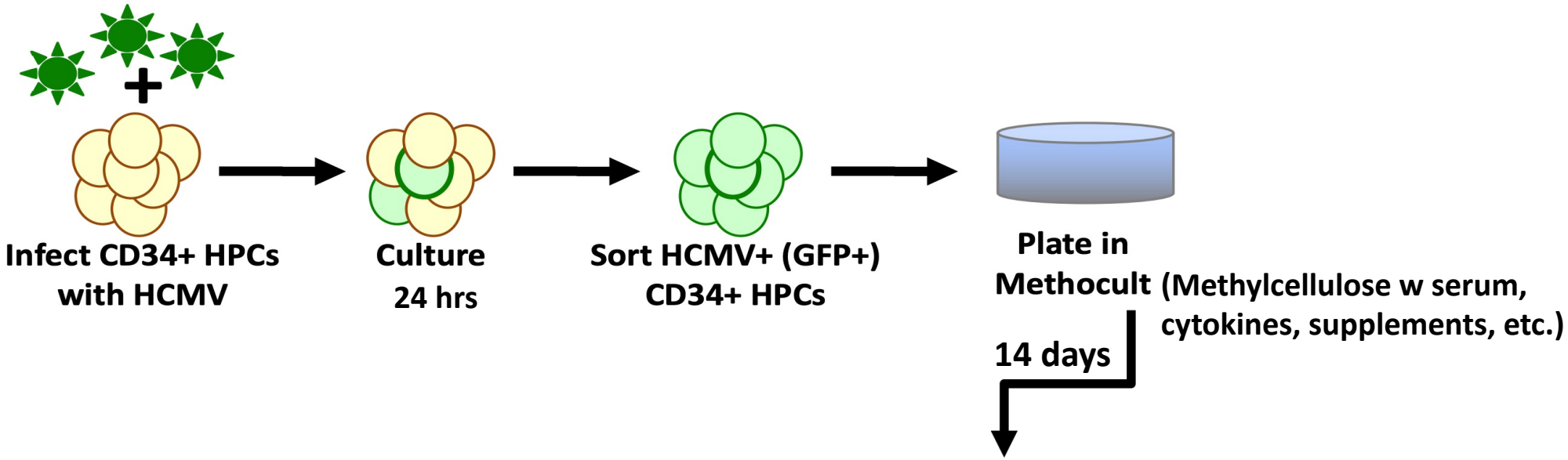
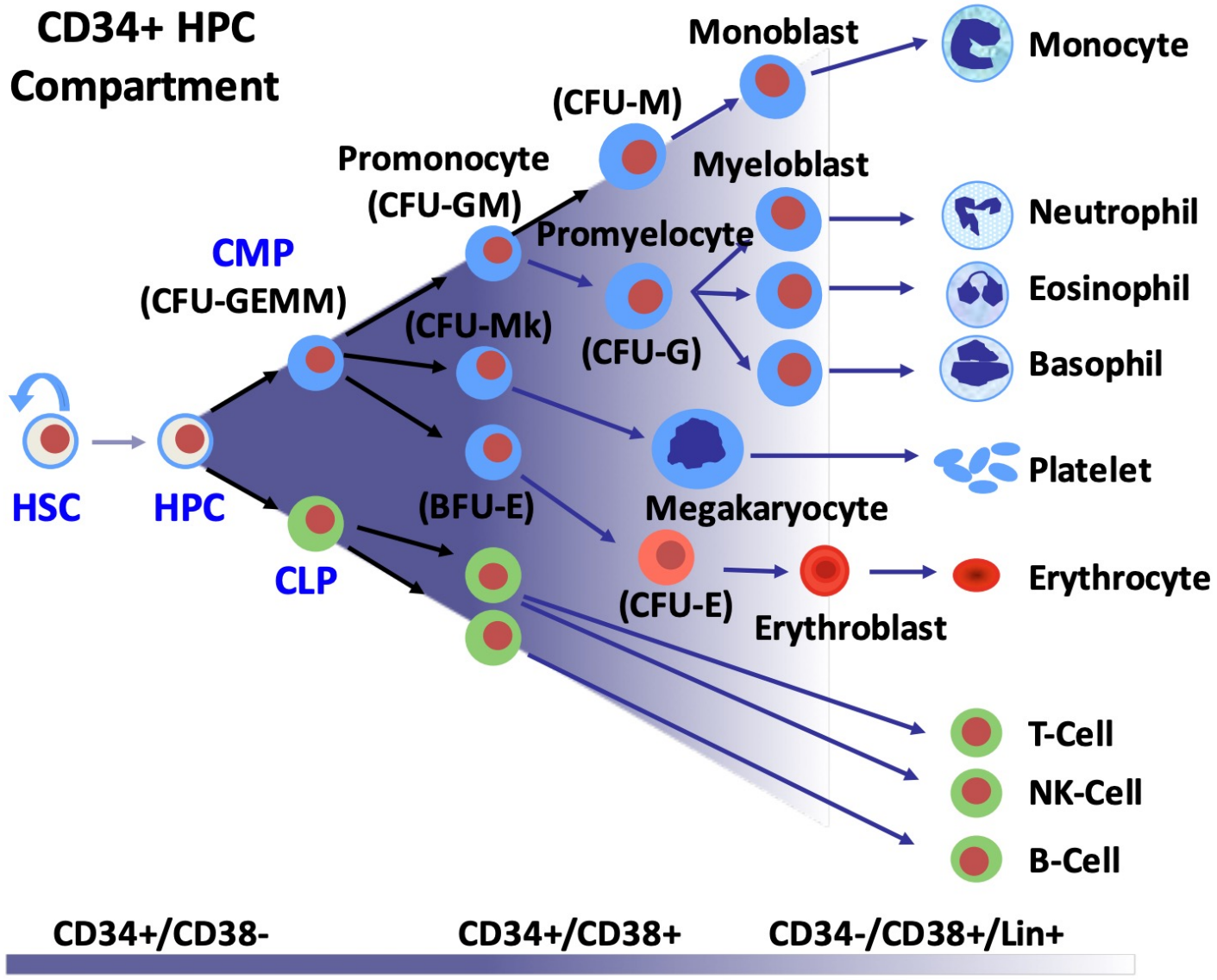
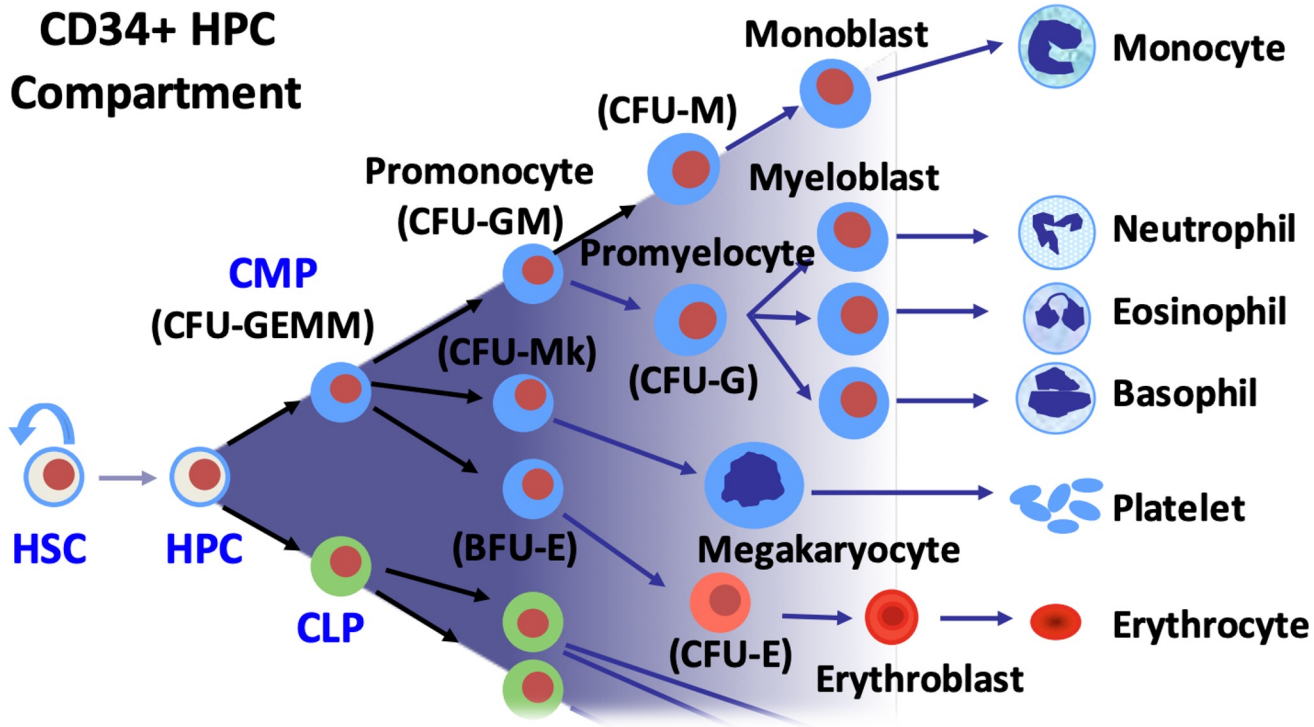
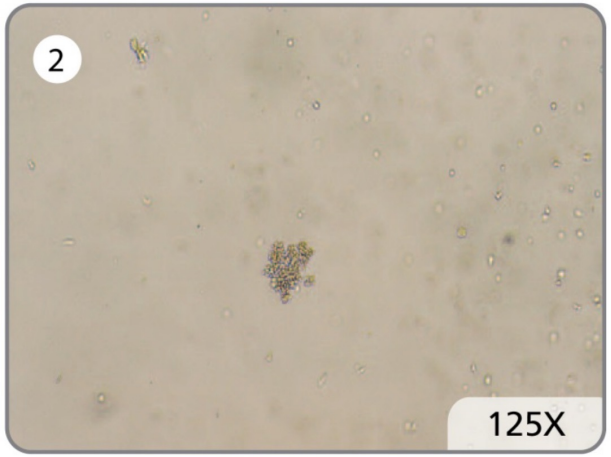


- **Count Progenitor Colony Types: CFU-E, BFU-E, CFU-GM, CFU-GEMM**
- **Compare Mock vs Infected CD34+ HPCs**

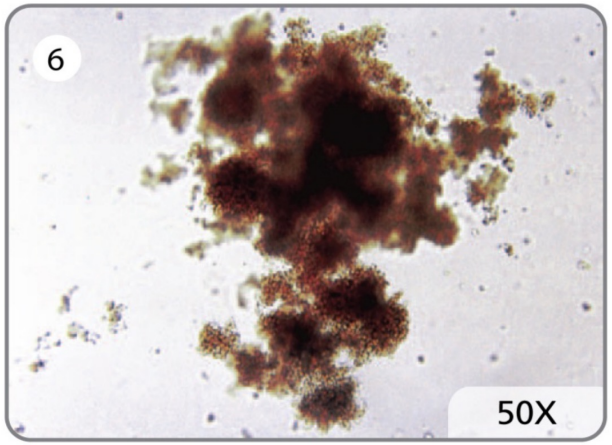
Human Hematopoiesis



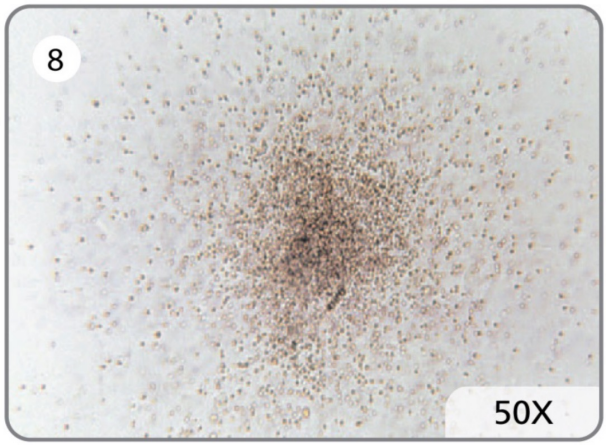




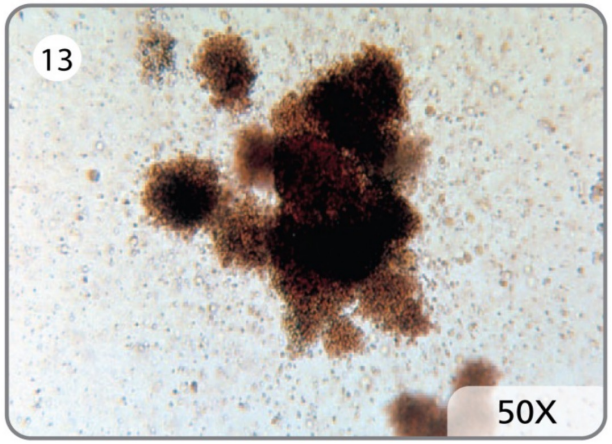
CFU-E



BFU-E



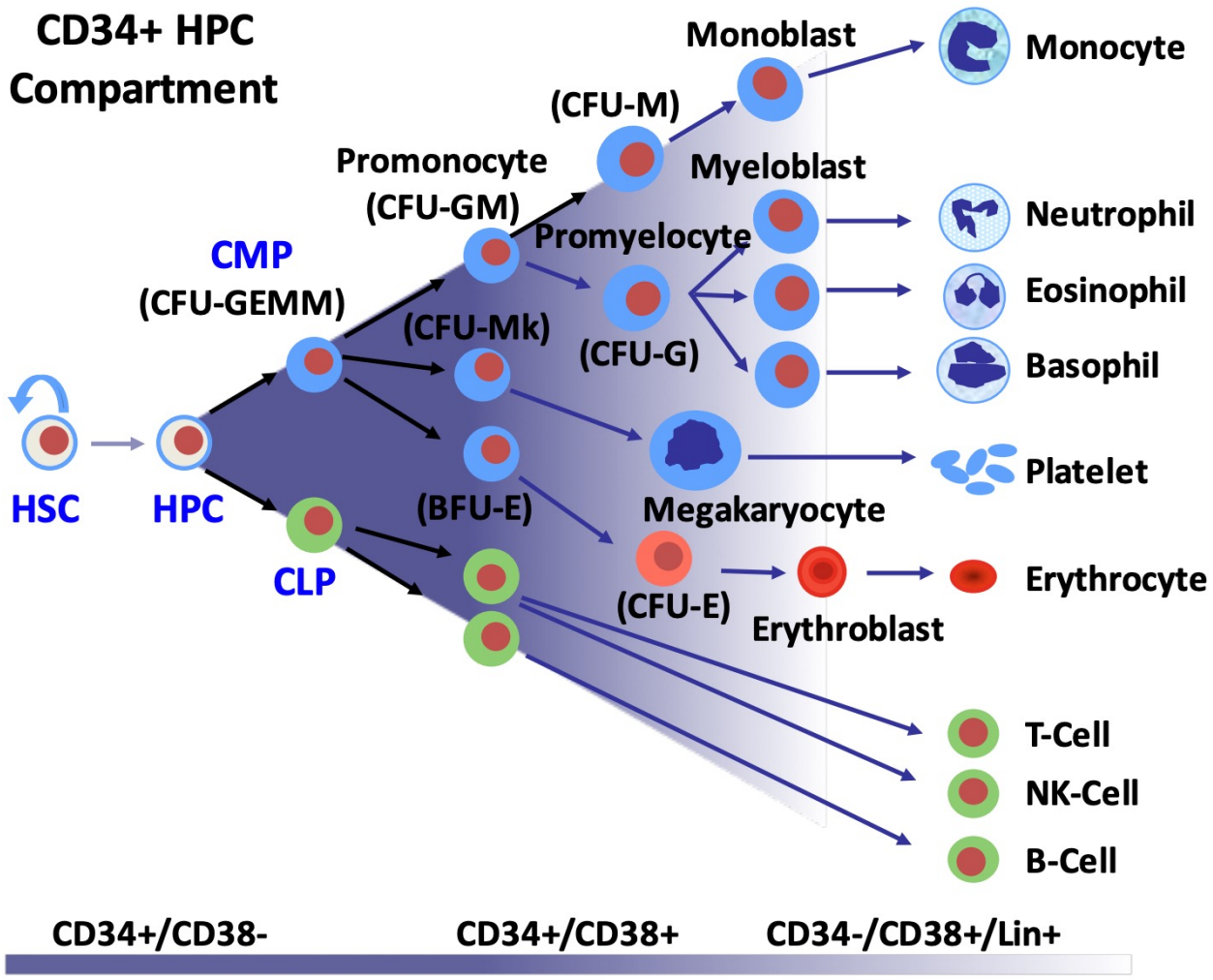
CFU-GM

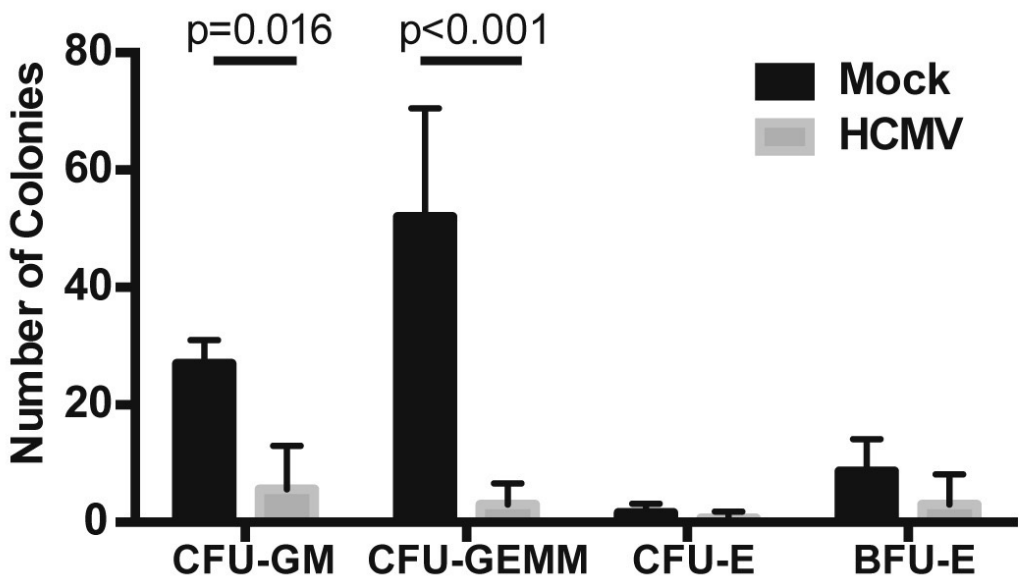


CFU-GEMM

Image courtesy of STEMCELL Technologies

WT HCMV causes myelosuppression of infected HPCs

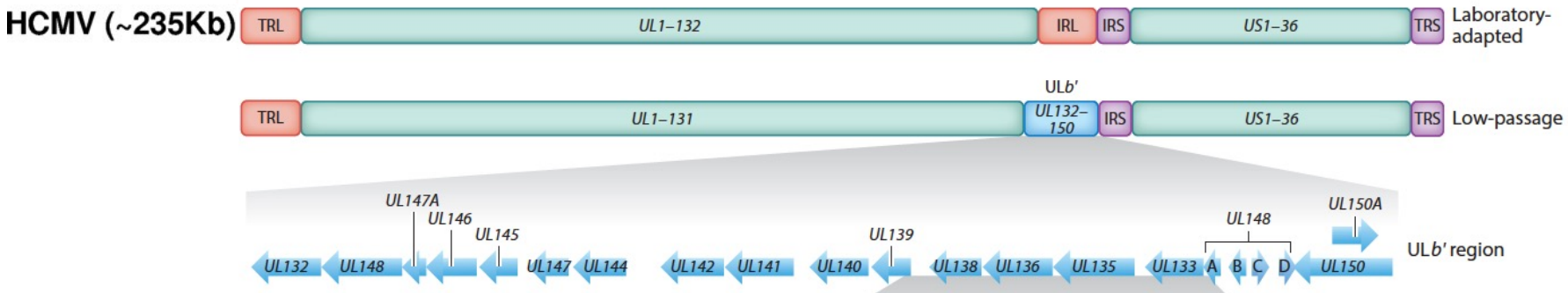




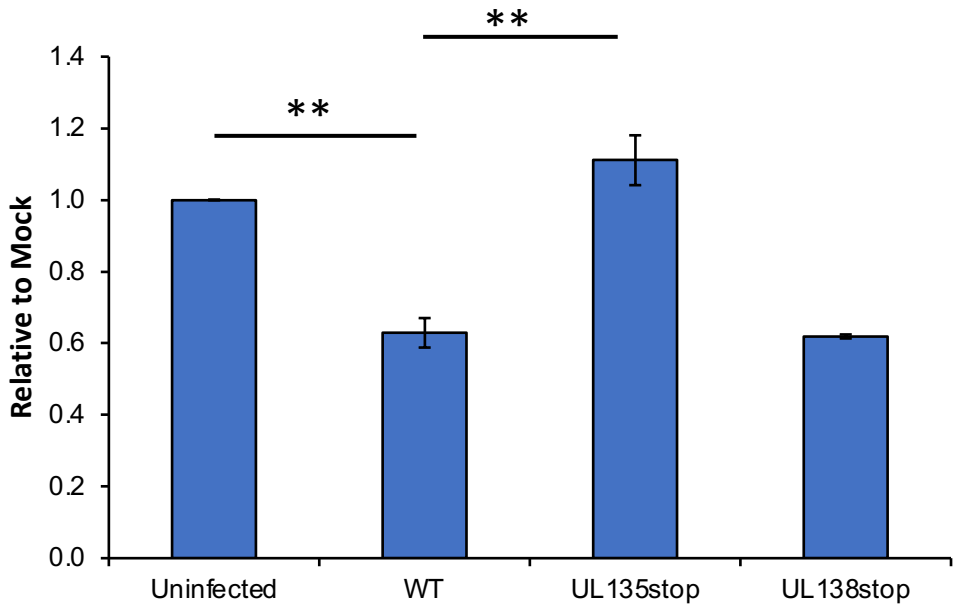
Hancock & Crawford et al, Cell Host & Microbe (2020)

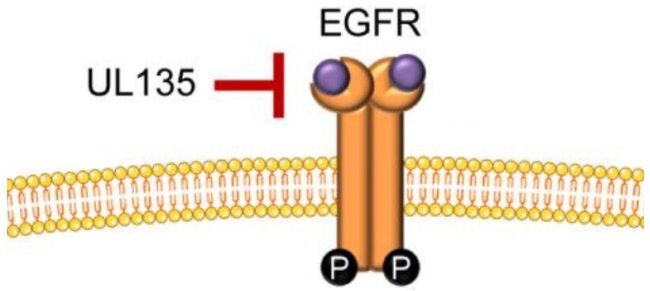
UL135 is required for HCMV-mediated myelosuppression

Goodrum, *Annual review of virology* 3 (2016)



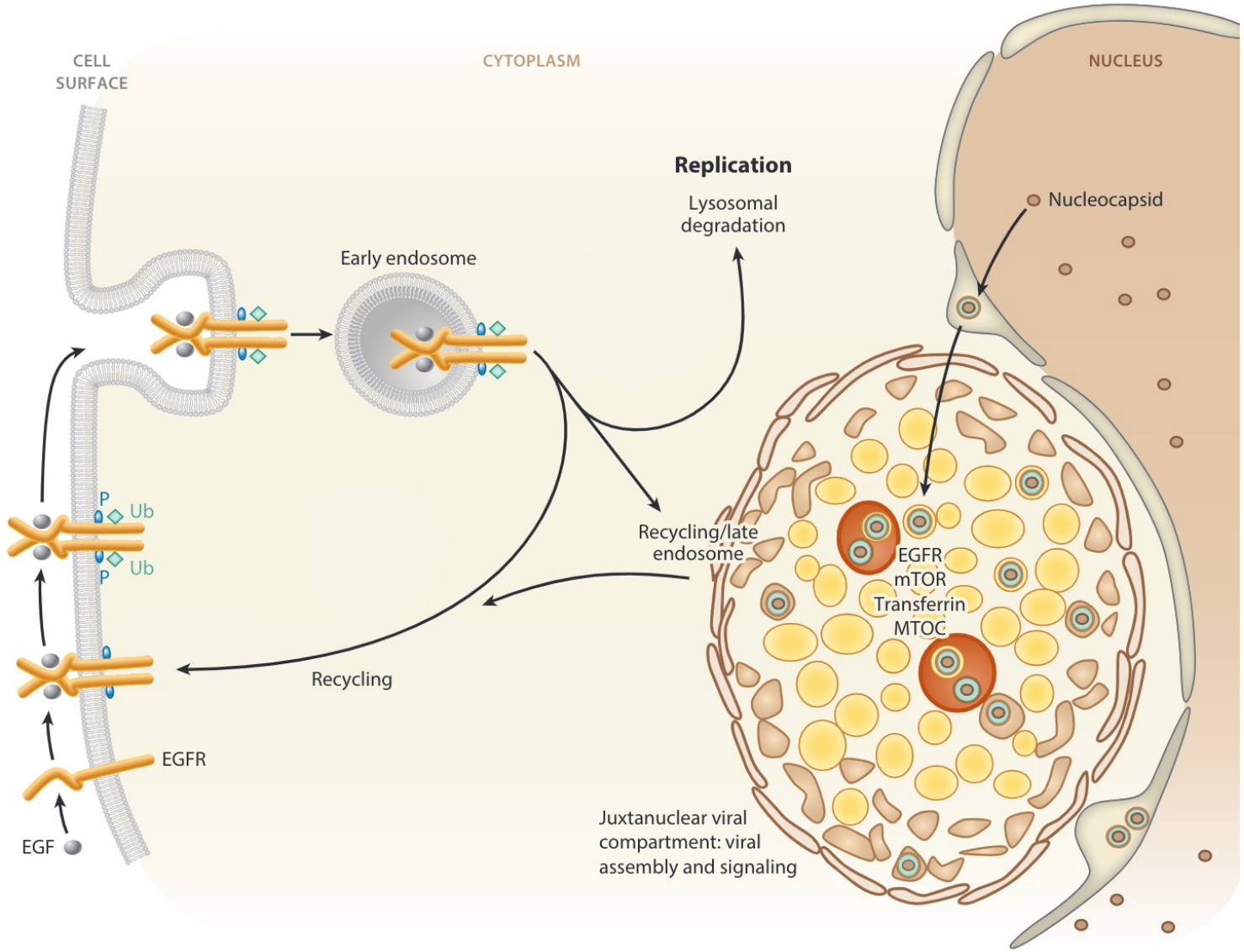
CFU-GM/GEMM





Buehler, unpublished

UL135 targets EGFR for internalization and degradation



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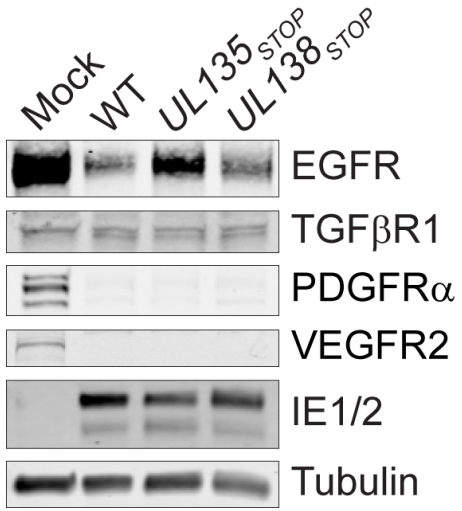
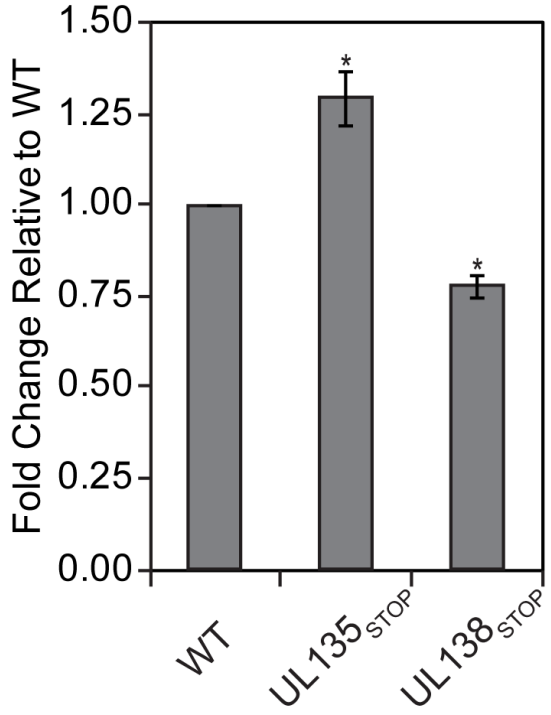
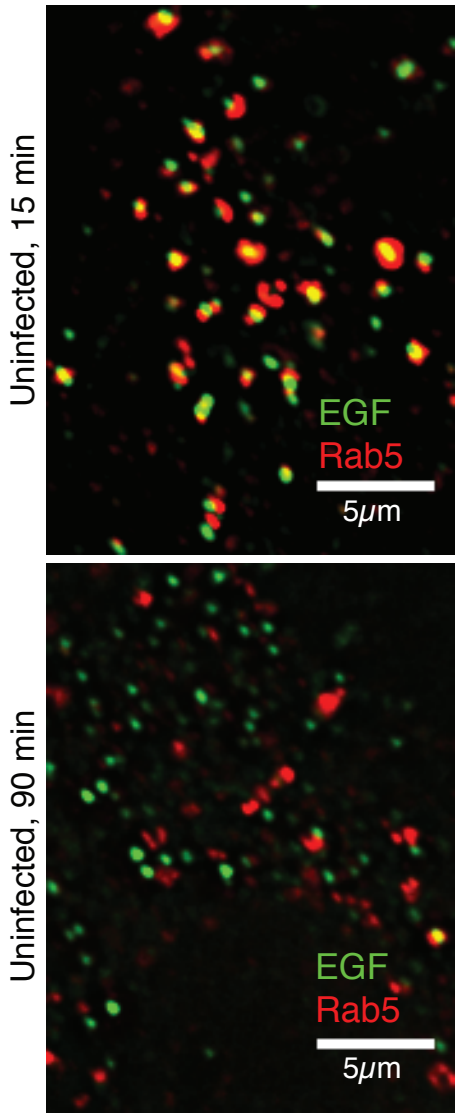
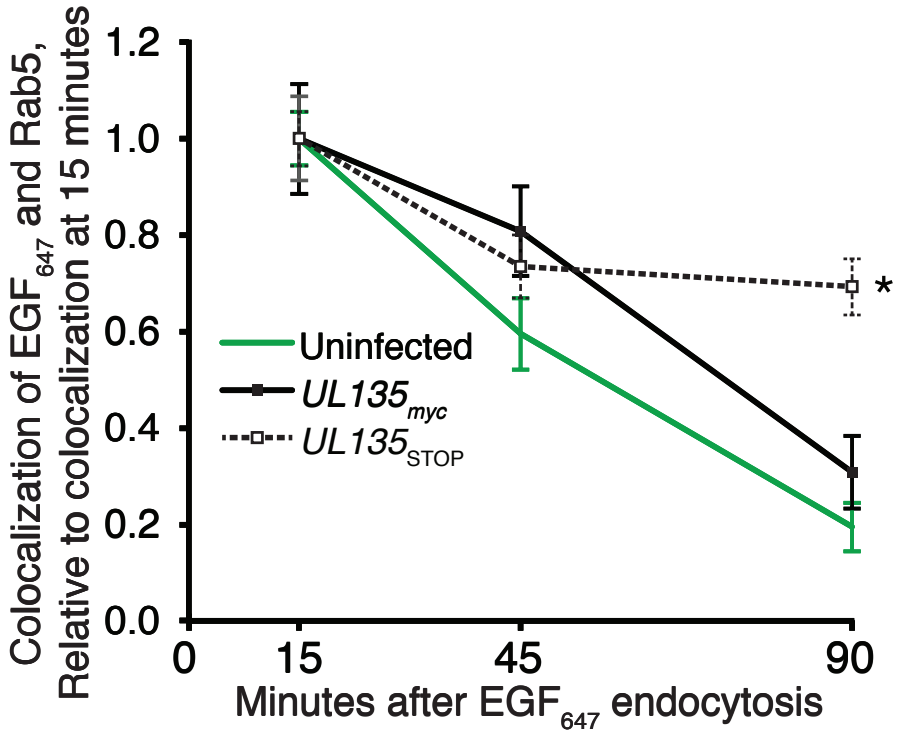
UL135 targets EGFR for internalization and degradation in fibroblasts







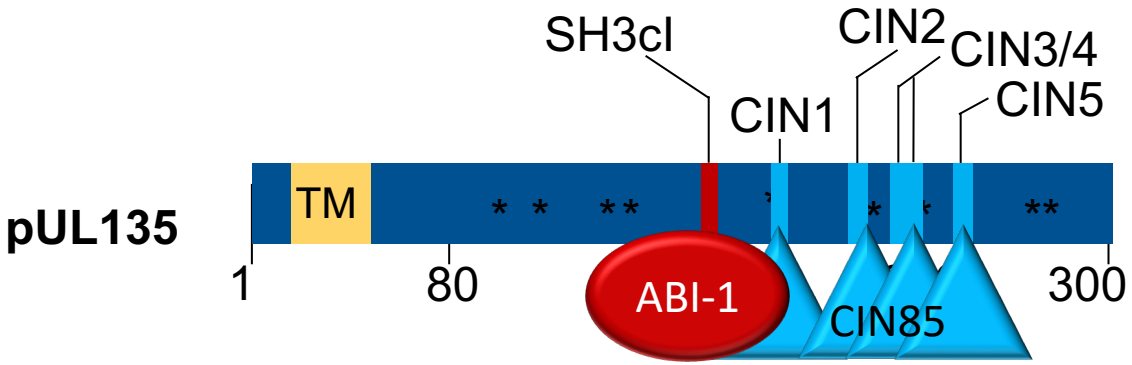


	Retention of EGF in Rab5 at 90 min
Uninfected	19%

Buehler et al. PLOS Path (2016)

Rak and Buehler et al., *J Virology* 2018

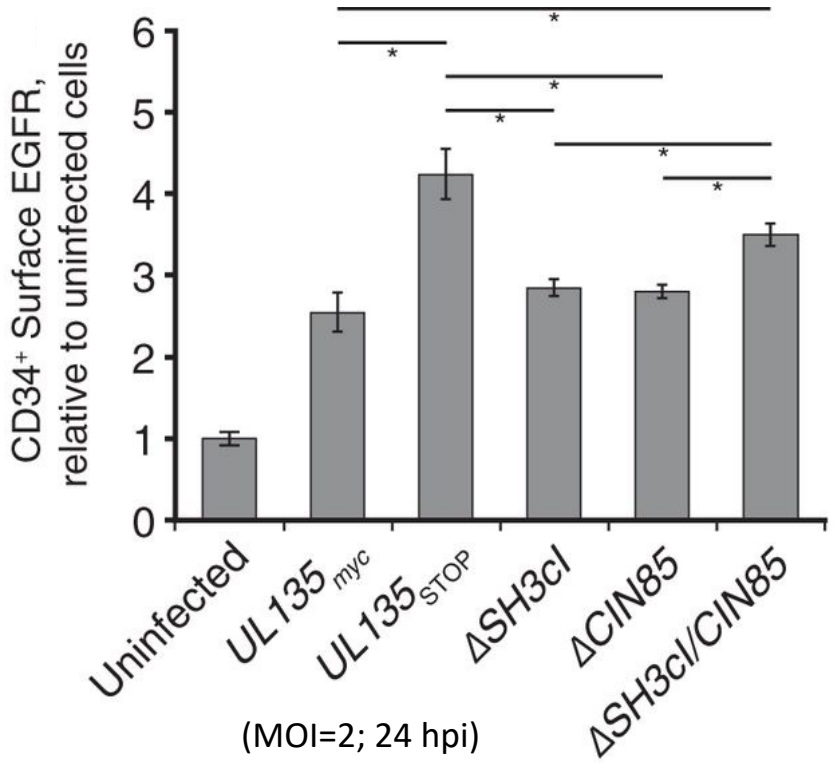
UL135 interaction with CIN85 and Abi-1 regulates EGFR surface levels in CD34⁺ HPCs



SH3 Ligand Class	Consensus Ligand	UL135 residues	WT Sequence	Mutated Sequence
SH3cl	(R/K)xxPxxP	187-193	KRPPTPP	**R**RPPTP**A**
CIN85	PxxxPR	209-214 238-243 251-256 254-259 277-282	PIPAPR PPVTPR PQKPPR PPRNPR PCPRPR	PIPA**AA** PPVT**AA** PQKP**AA** P**AA**N**AA** PCPR**AA**

Adaptor proteins:

- CIN85 & Abi1 recruit
  - Clathrin adapter AP2
  - Endophilins (BAR domain)
  - Cbl: E3 ubiquitin ligase



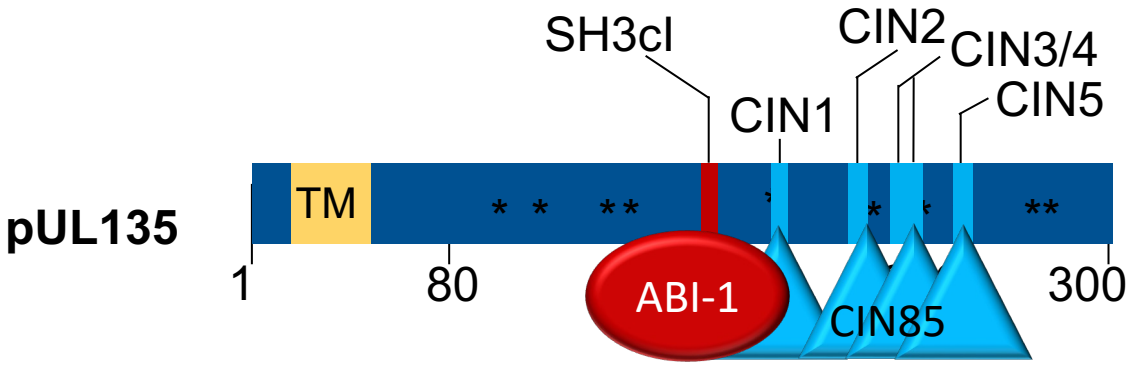
EGFR surface level by EGF_{647} binding analysis by flow

- Single domain mutants are similar to 135_{myc} or WT
- Double domain mutant is more similar to 135_{stop}

➔ Both Abi-1 and CIN85 interactions are necessary for regulation of EGFR surface level

Rak et al. JVI (2018)

UL135 domain mutant virus myelosuppression phenotype implicates the role of EGFR signaling in HCMV-mediated myelosuppression



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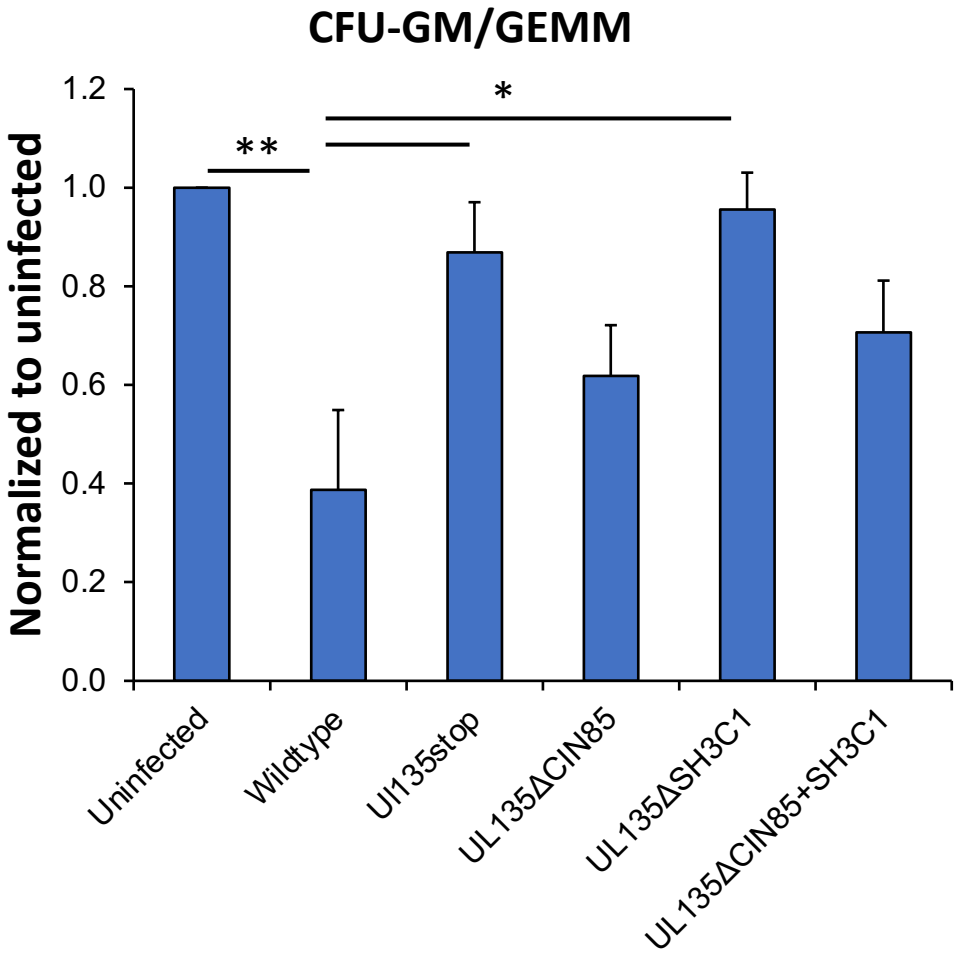
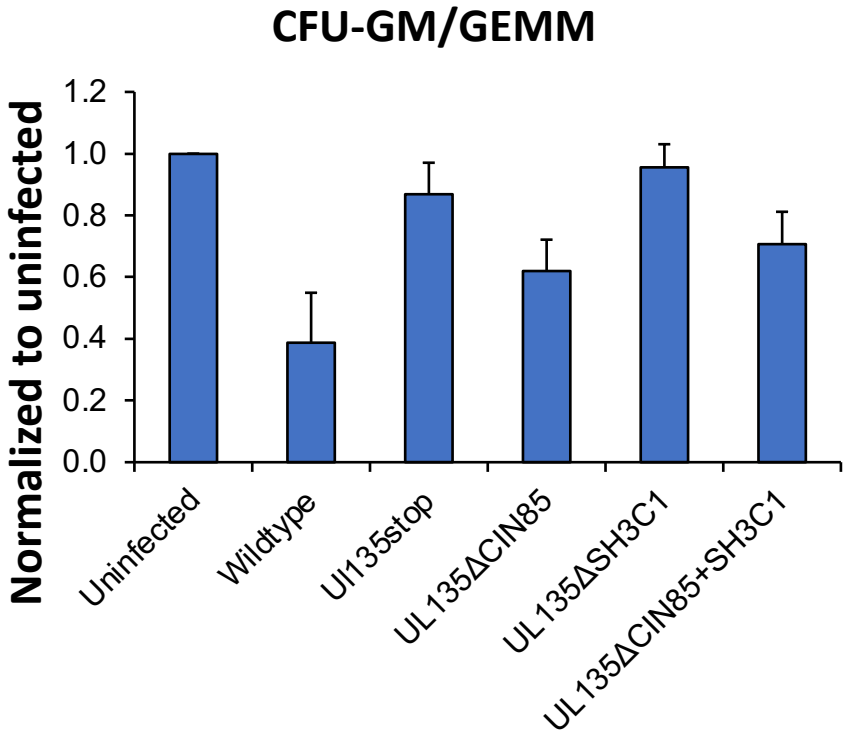
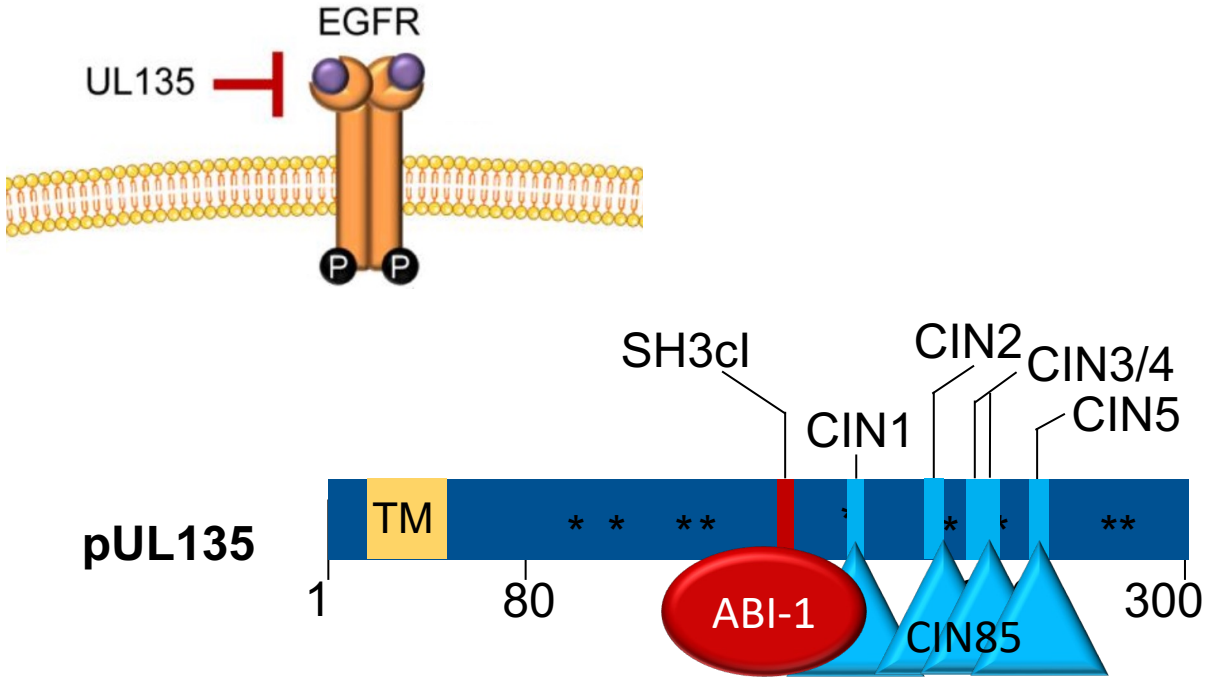


Buehler, unpublished

Summary





- HCMV causes myelosuppression in infected CD34⁺ hematopoietic progenitor cells
- HCMV lacking UL135 fails to cause myelosuppression in infected HPCs
- UL135 downregulates EGFR expression and surface levels by interacting with host adaptor proteins CIN85 and Abi-1
- Virus with UL135 mutation in host interaction domains also fails to cause myelosuppression in infected HPCs

Central question: how does UL135 modulate CD34^{+} HPC signaling to induce myelosuppression?

1. How do UL135 interactions with CIN85 & Abi-1 affect EGFR signaling in HPCs during infection?
2. What downstream signaling pathway(s) and molecular components are important for HPC myeloid lineage-specific differentiation?
3. What are the transcription factors driving myeloid differentiation that are involved in HCMV-induced myelosuppression of HPCs?

Acknowledgement

Goodrum lab

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- **Jason Buehler, PhD**
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- Kristen Maness
- Pierce Longmire
- David Tafoya
- Liz Alolod

Thank you!
Questions?

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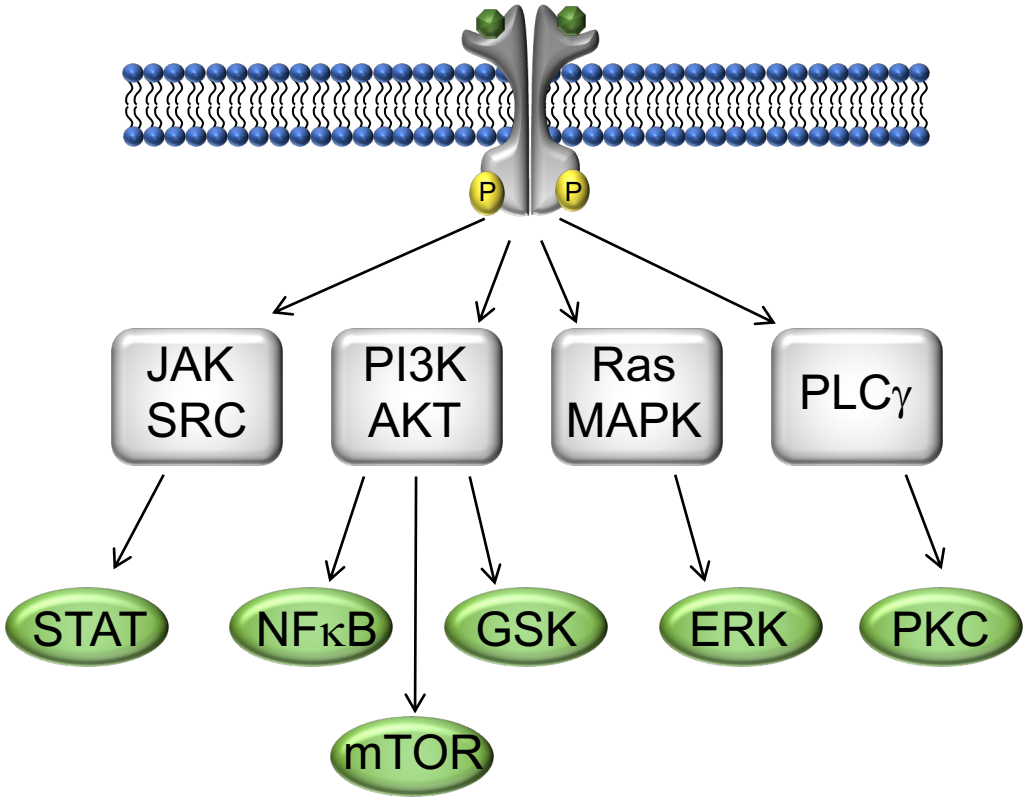
1. How do UL135 interactions with CIN85 & Abi-1 affect EGFR signaling in HPCs during infection?

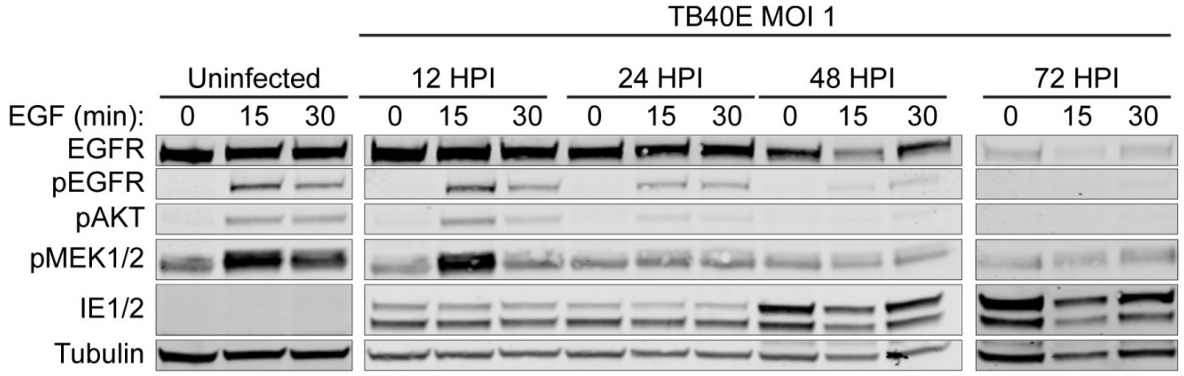
- Rationale/premise:
  - UL135-host interactions are important for HCMV-induced myelosuppression
  - UL135 through host interactors downregulate EGFR expression and surface levels

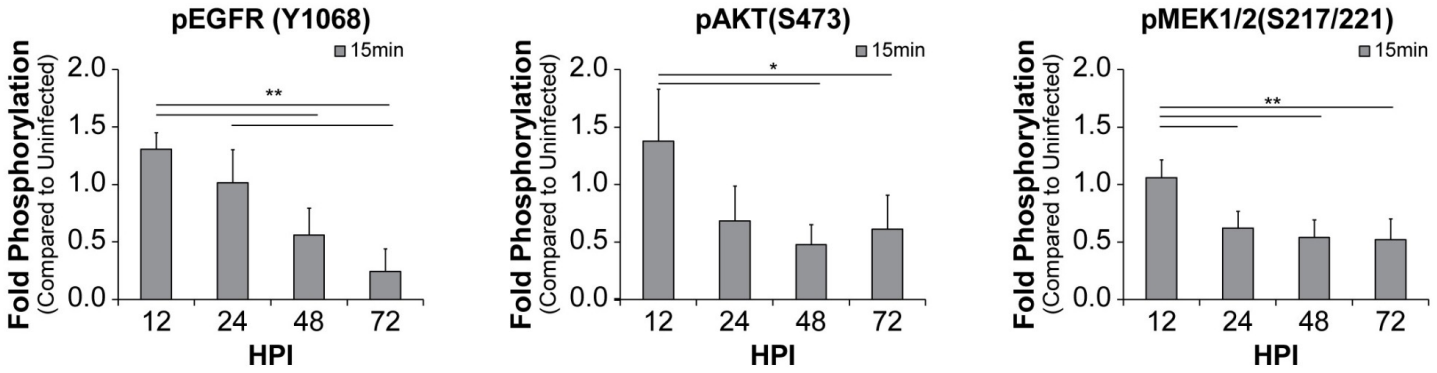


Survival Proliferation Oncogenesis	Angiogenesis Survival Tumorigenesis Protein Synthesis Cytoskeleton	Gene expression Cell Cycle	Differentiation Tumorigenesis Apoptosis

1. How do UL135 interactions with CIN85 & Abi-1 affect EGFR signaling in HPCs during infection?

- WT vs UL135stop vs UL135ΔCIN85/Abi-1
- MRC-5 (fibroblasts): Serum-starve and pulse with EGF for 15 minutes, time points 12, 24, 48, 72 hpi
- Phospho-blot/phospho-flow to measure EGFR downstream signaling in MRC-5 & $CD34^{+}$ HPCs





- Expect:
  - EGFR signaling will be sustained in UL135stop vs UL135ΔCIN85/Abi-1 compared to WT
- Alternatives:
  - Abi-1 & WAVE2 complex in cytoskeleton remodeling and immune synapse formation
  - Abi-1 & Src family kinases, STAT3, and NF-kB signaling

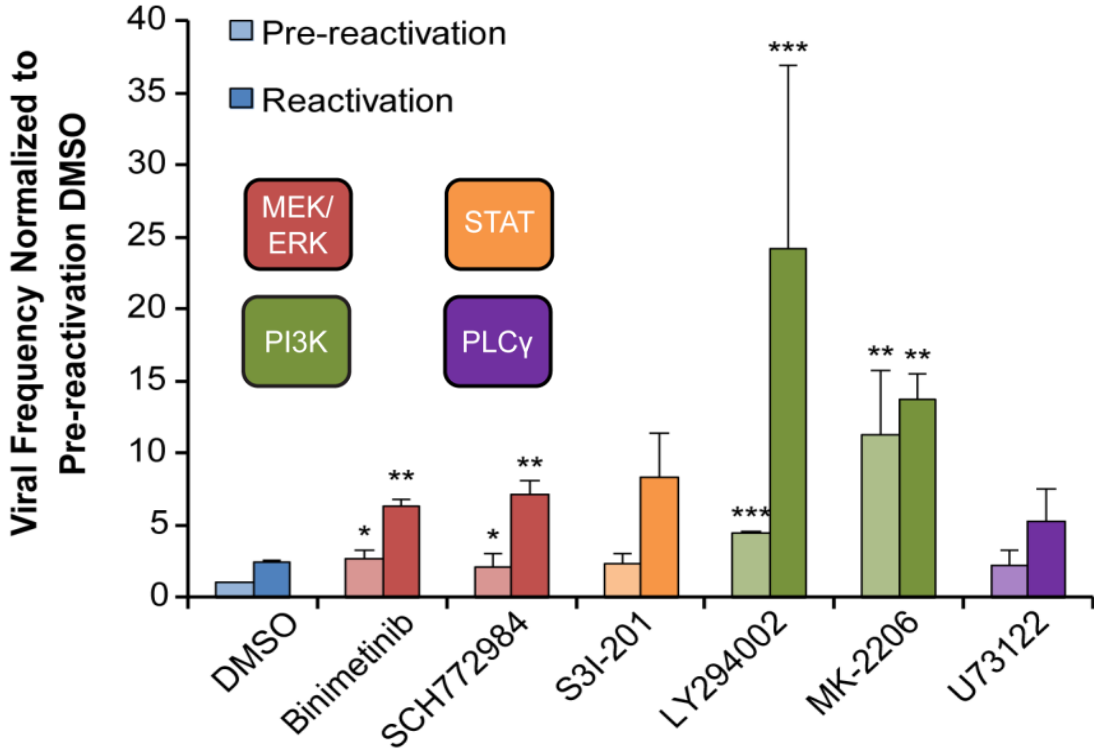
Buehler et al. PLoS Path. (2019)

2. What downstream signaling pathway(s) and molecular components are important for HPC myeloid lineage-specific differentiation?

- Rationale/premise:
  - Identify host pathways specific host proteins important for myelopoiesis that might be targeted by UL135

2. What downstream signaling pathway(s) and molecular components are important for HPC myeloid lineage-specific differentiation?

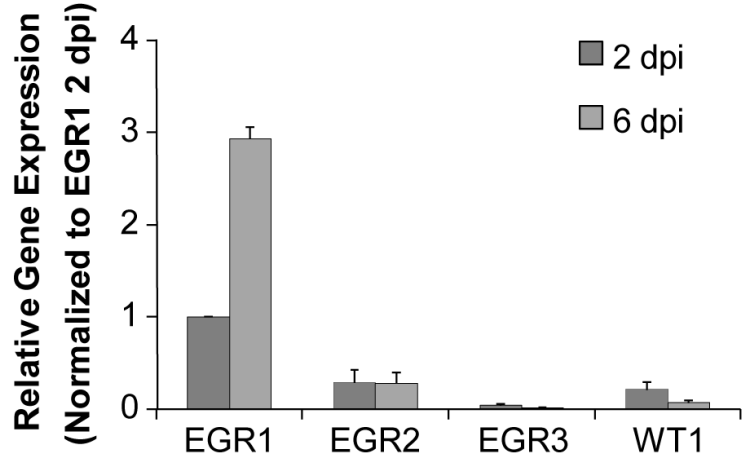
- CFU assay for $CD34^+$ HPCs
  - HPCs treated with various EGFR signaling & other pathways drug targets
    - EGFR: Gefitinib
    - PI3K: LY294002
    - AKT: MK-2206
    - MEK: Binimetinib
    - ERK: SCH772984
    - STAT: S3I-201
    - PLCy: U73122
- Expect:
  - Inhibition of pathways important for myelopoiesis will lead to myelosuppression
- Alternatives:
  - Full Moon Cell Signaling Phospho antibody array
  - Specific downstream targets (inhibitor/shRNA)



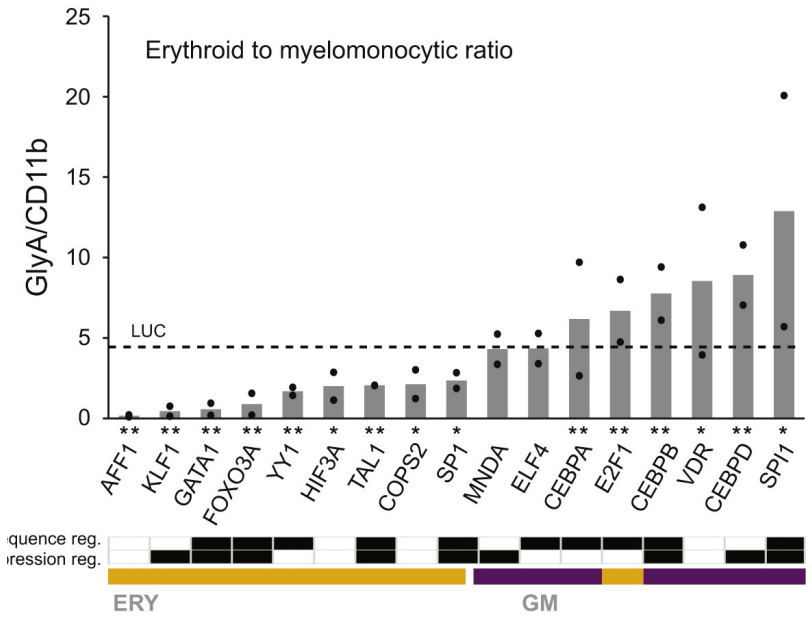
Buehler et al. PLoS Path. (2019)

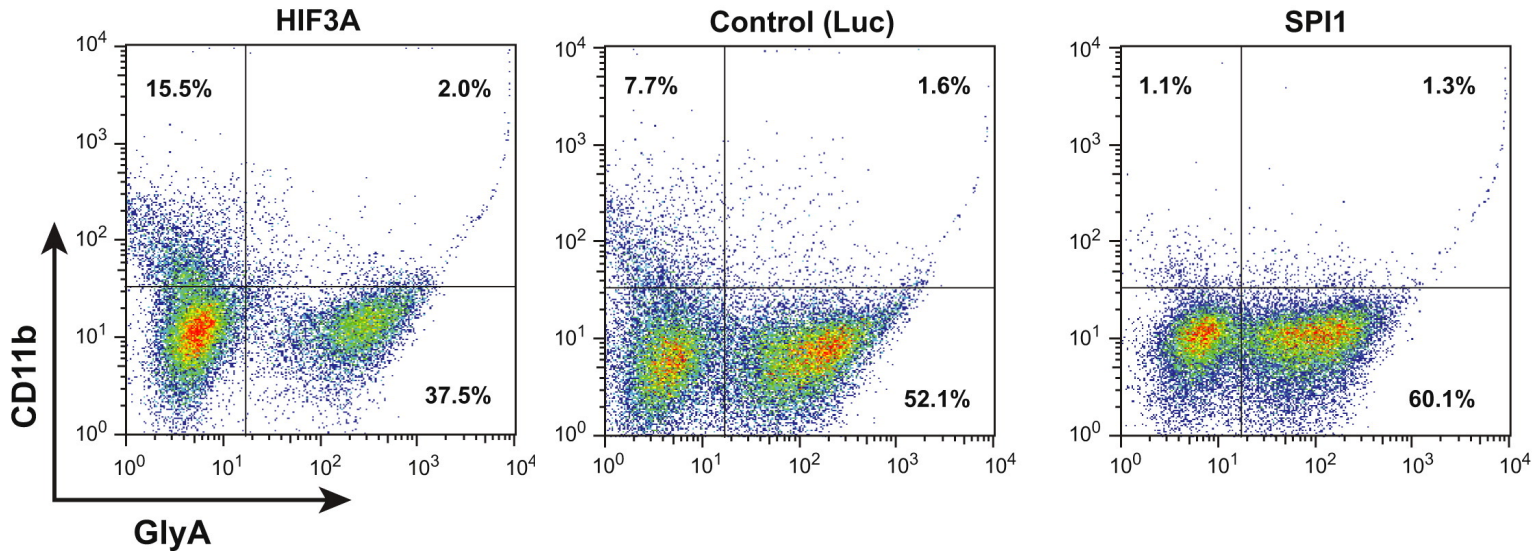
3. What are the transcription factors driving myeloid differentiation that are involved in HCMV-induced myelosuppression of HPCs?

- Myeloid lineage transcription factors
  - CEBPα, β, δ
  - PU.1 (SPI1)
- Measuring transcripts and protein levels
  - WT, UL135stop, UL135ΔCIN85/Abi-1
  - Expect lower levels in WT (myelosuppressed)
- Alternatives:
  - RNA-seq of WT vs UL135stop in CD34⁺ HPCs and THP-1 cells



Buehler et al. PLoS Path. (2019)





Novershtern & Subramanian et al. Cell (2011)

Central question: how does UL135 modulate CD34^{+} HPC signaling to induce myelosuppression?

1. How do UL135 interactions with CIN85 & Abi-1 affect EGFR signaling in HPCs during infection?
2. What downstream signaling pathway(s) and molecular components are important for HPC myeloid lineage-specific differentiation?
3. What are the transcription factors driving myeloid differentiation that are involved in HCMV-induced myelosuppression of HPCs?

Thank you!
Questions?