

# A robust and flexible baculovirus-insect cell system for AAV vector production with improved yield, capsid ratios and potency

Abstract #471



Yoko Marwidi, Hoang-Oanh Nguyen, David Romero-Santos, Sumita Bhardwaj, Gabriel Ernie, Garrett Lew, Michael Trias, Jada Padilla, Hung Tran, Kathleen Meyer, Richard Surosky, and Alex Ward

Sangamo Therapeutics Inc., 501 Canal Blvd, Richmond, CA 94804, USA

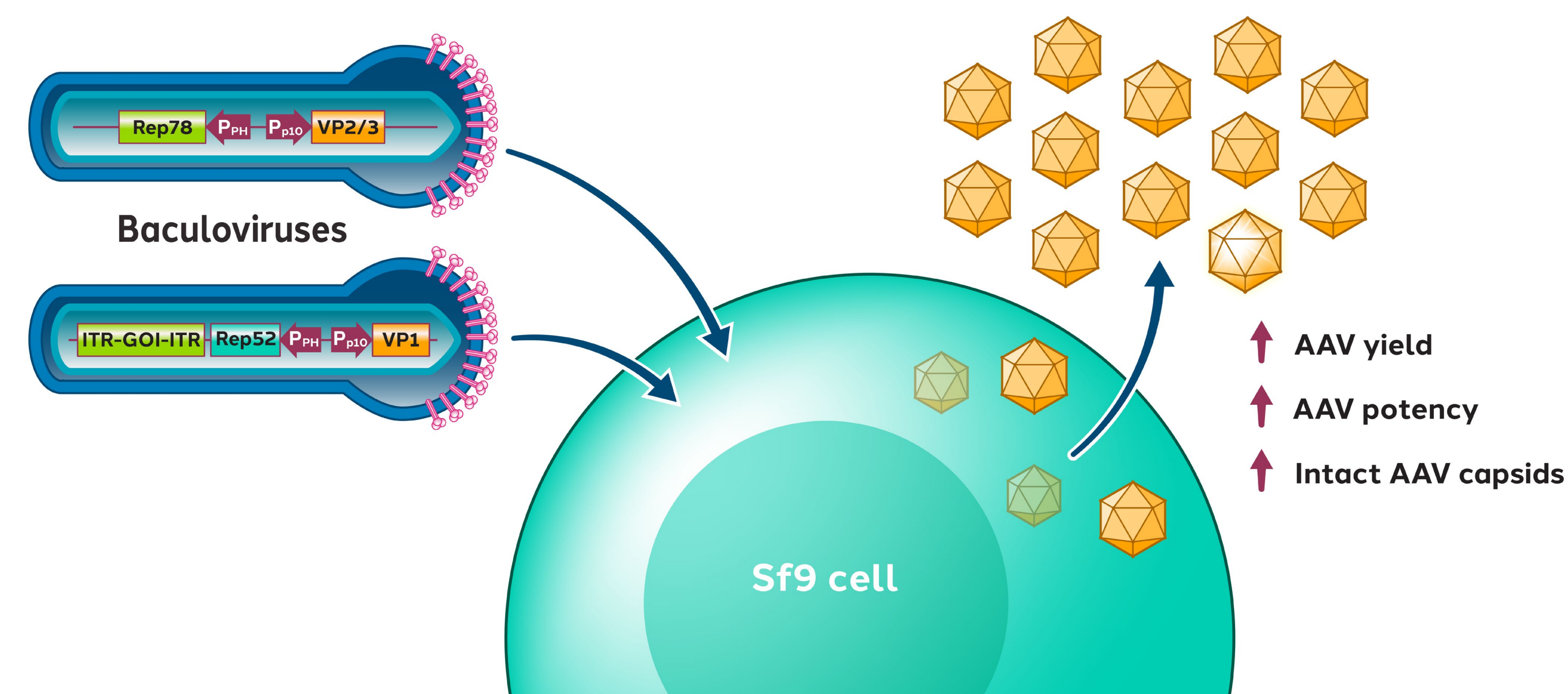
## Introduction

Manufacturing of recombinant adeno-associated viruses (rAAV) for gene and cell therapy applications has increased significantly and spurred development of improved mammalian and insect cell-based production systems. We developed a baculovirus-based insect cell production system- the Sangamo (SGMO) Helper- with a novel gene architecture and greater flexibility to modulate the expression level and content of individual Rep and Cap proteins. In addition, we incorporated modifications to the rAAV6 capsid sequence that improves yield, capsid integrity and potency:

- Production of rAAV6 using the SGMO Helper has improved yields compared to the Bac-RepCap helper from the Kotin lab.
- SGMO Helper-derived rAAV6 is resistant to a previously described proteolytic cleavage unique to baculovirus-insect cell production systems and has improved capsid ratios and potency, in vitro and in vivo, compared to rAAV6 produced using Bac-RepCap.
- AAV production using the SGMO Helper is scalable using bioreactors, with similar improvements in yield, capsid ratio and in vitro potency.

Our studies demonstrate that the SGMO Helper is an improved platform for rAAV manufacturing to enable delivery of cutting-edge gene and cell therapies.

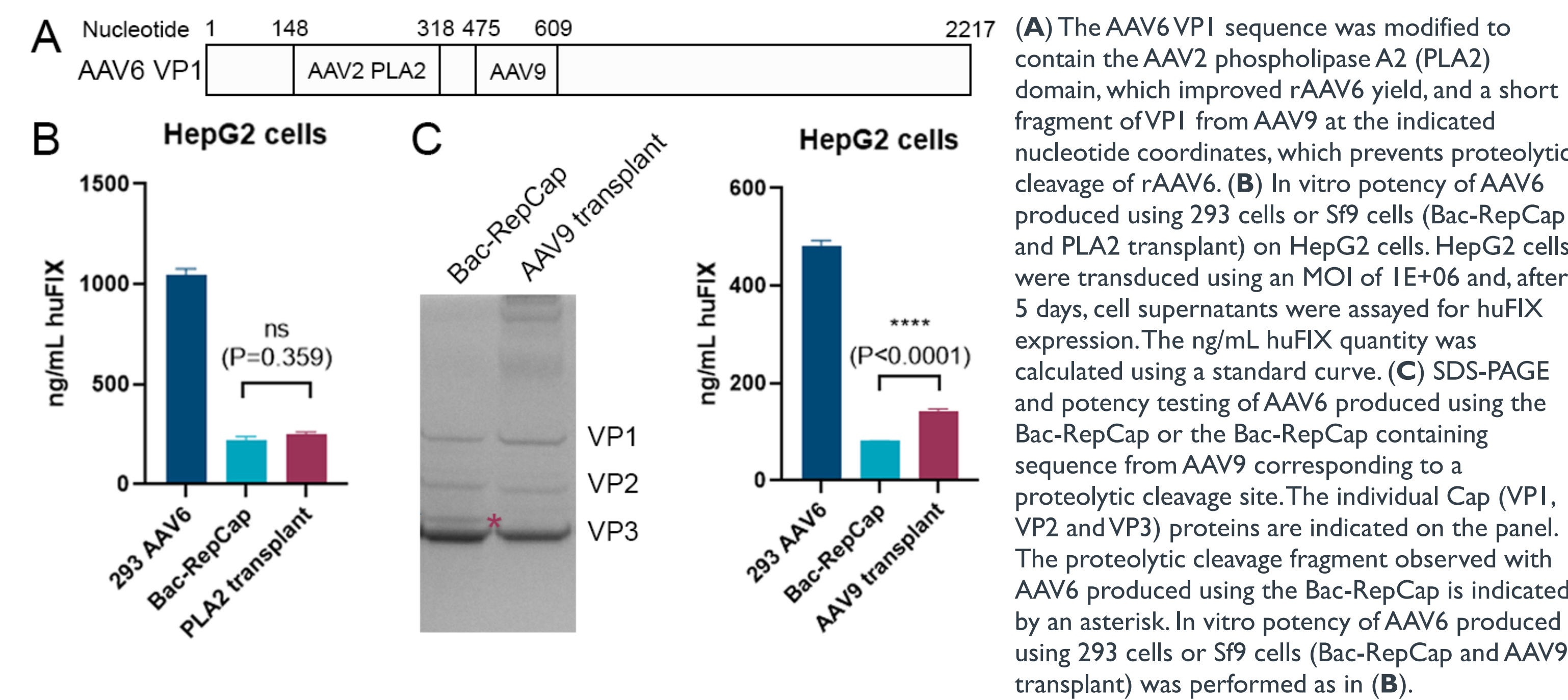
## Gene Architecture of the SGMO Helper



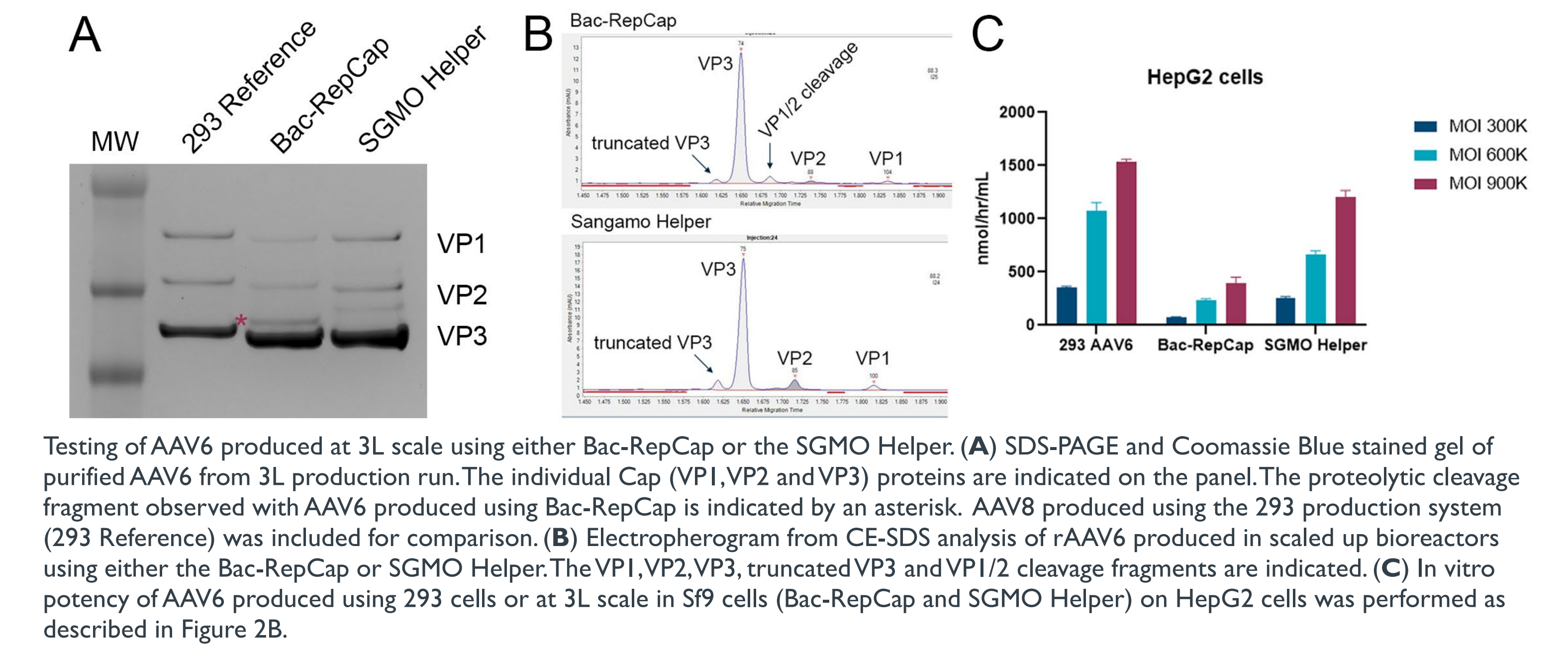
## Production of rAAV6 using the SGMO Helper

Experiment	Sample	Purified AAV titer (vg/mL)	Purified AAV yield (vg)	Harvest yield (vg/mL culture)	VP1	VP2	VP3
1	Bac-RepCap	2.23E+13	2.45E+13	1.23E+11	1	2.1	45
	SGMO Helper	5.99E+13	8.99E+13	4.50E+11	1	1.1	21
2	Bac-RepCap	2.25E+13	2.70E+13	1.35E+11	1	2.1	60
	SGMO Helper	7.97E+13	1.20E+14	6.00E+11	1	1.3	24
3	Bac-RepCap	9.46E+12	9.46E+12	4.73E+10	1	2.1	67
	SGMO Helper	6.21E+13	8.69E+13	4.35E+11	1	1	19
4	Bac-RepCap	1.83E+13	1.65E+13	8.25E+10	1	1.8	60
	SGMO Helper	8.40E+13	1.09E+14	5.45E+11	1	0.8	17
Mid-scale production	Bac-RepCap	3.56E+13	3.98E+14	2.65E+11	1	1.7	82
	SGMO Helper	4.85E+13	9.89E+14	6.59E+11	1	1.2	24
In vivo study	Bac-RepCap	1.37E+13	3.01E+13	1.51E+11	1	1.5	59
	SGMO Helper	3.47E+13	1.15E+14	5.75E+11	1	0.9	27

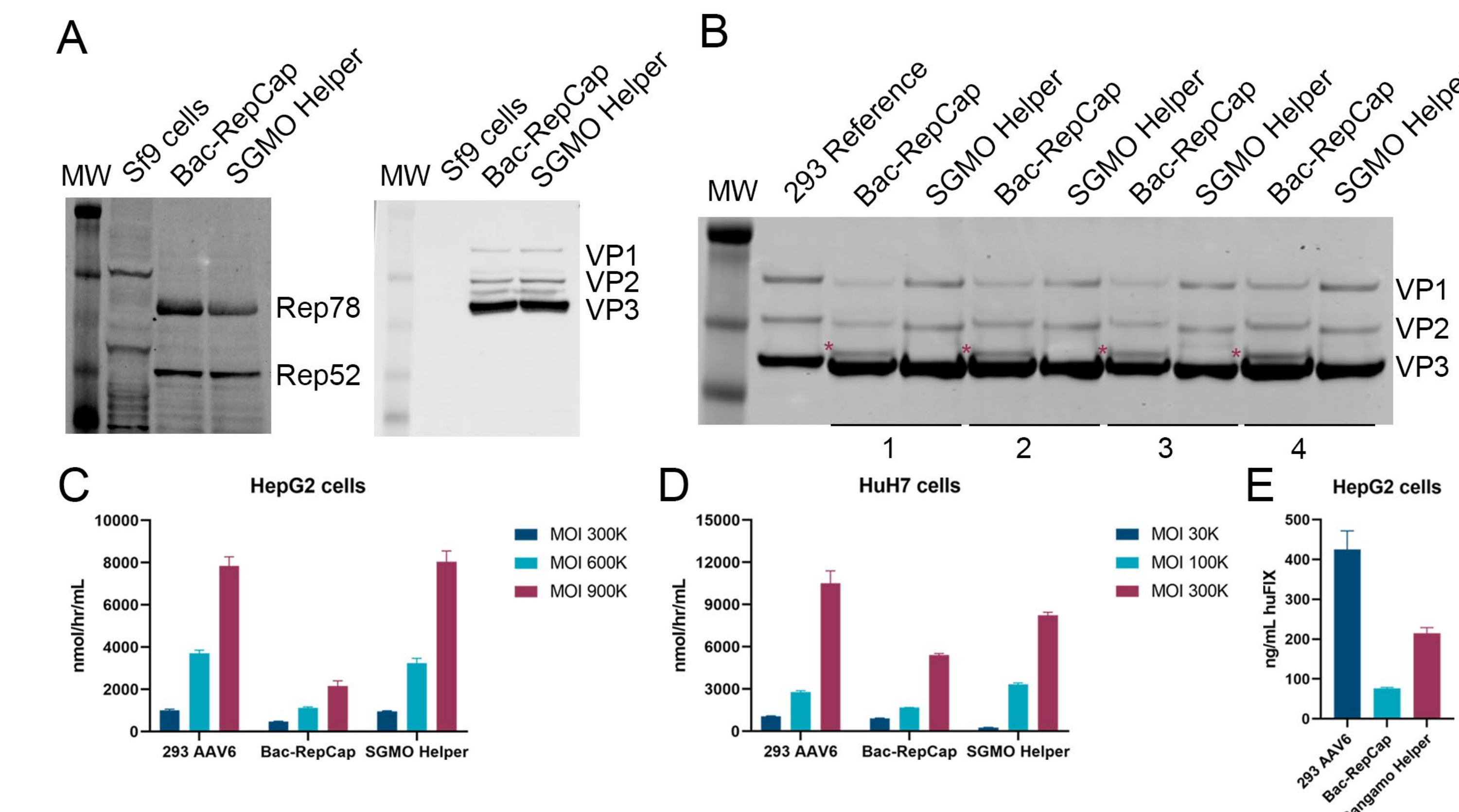
## Modifications to rAAV6 capsids produced in insect cells



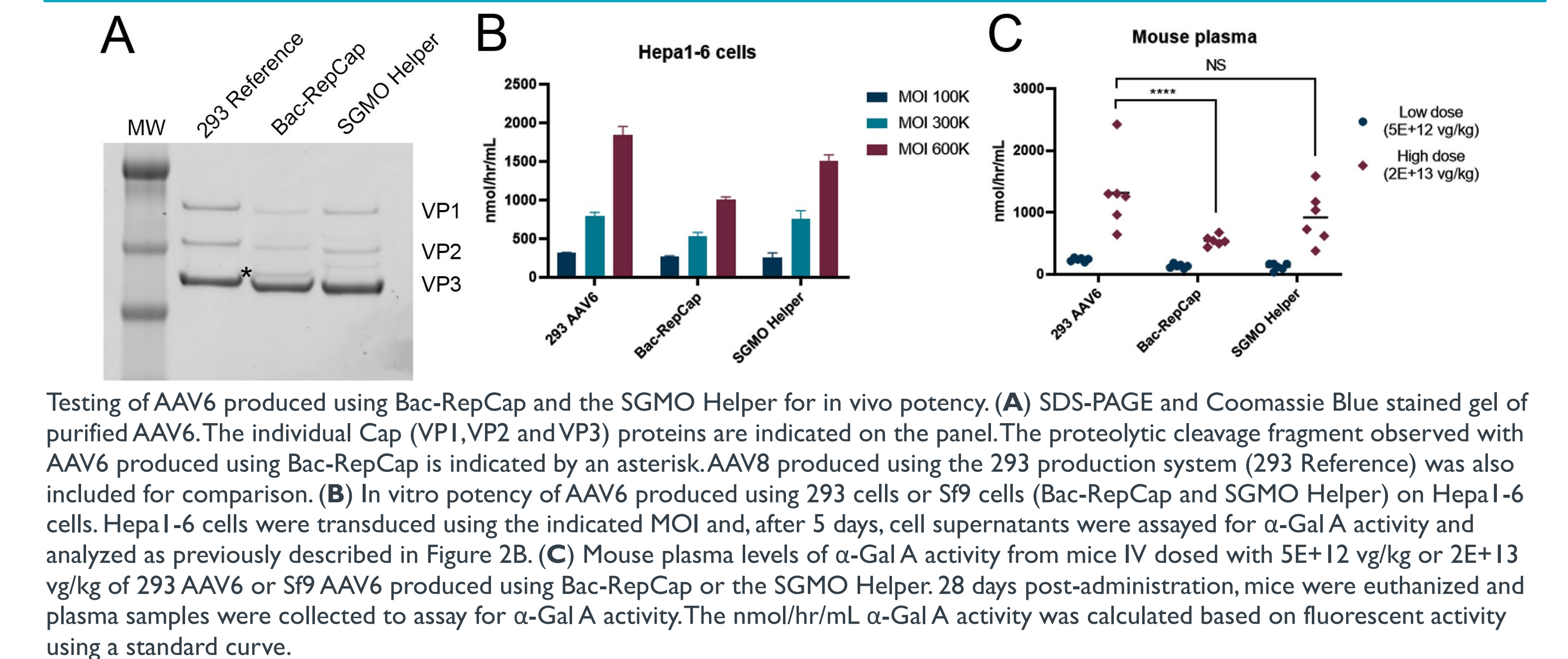
## Testing rAAV6 from scaled-up production using the SGMO Helper



## Testing rAAV6 produced using the SGMO Helper



## In vivo testing of rAAV6 produced using the SGMO Helper



## Conclusion

- Production of rAAV6 using the SGMO Helper has improved yields compared to the Bac-RepCap helper from the Kotin lab.
- SGMO Helper-derived rAAV6 is resistant to a previously described proteolytic cleavage unique to baculovirus-insect cell production systems and has improved capsid ratios and potency, in vitro and in vivo, compared to rAAV6 produced using Bac-RepCap.
- AAV production using the SGMO Helper is scalable using bioreactors, with similar improvements in yield, capsid ratio and in vitro potency.

## References

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