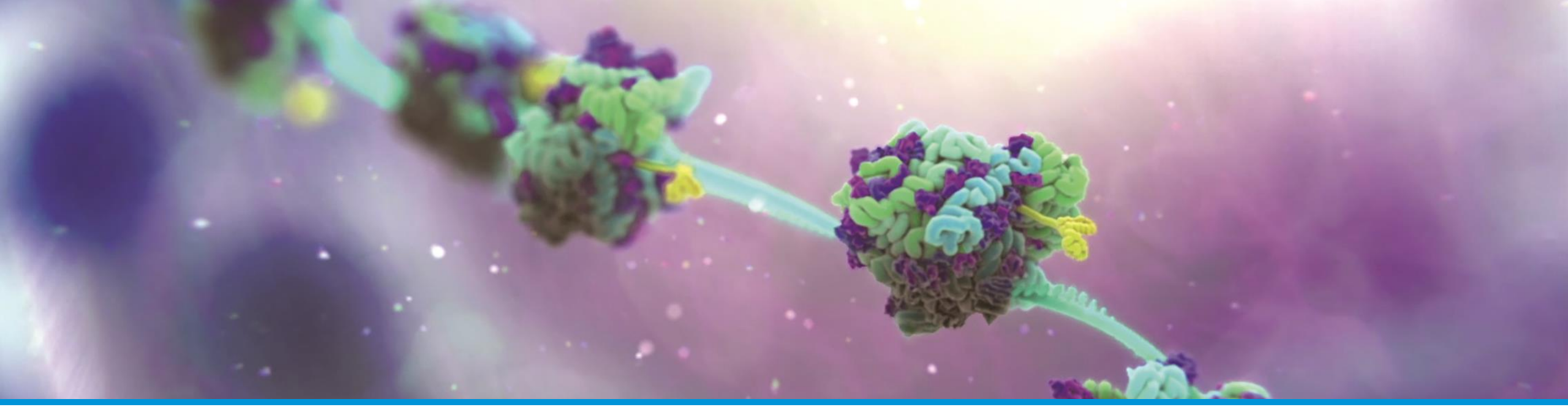

iMSCs Derived from mRNA-Engineered B2M-KO iPSCs Exhibit Enhanced Immunosuppressive Activity and Stealthing Features

Raven Dance Hinkel, Elizabeth Belcher, Christopher B. Rohde, Matthew Angel, Kyle M. Garland

Factor Bioscience Inc., Cambridge, MA

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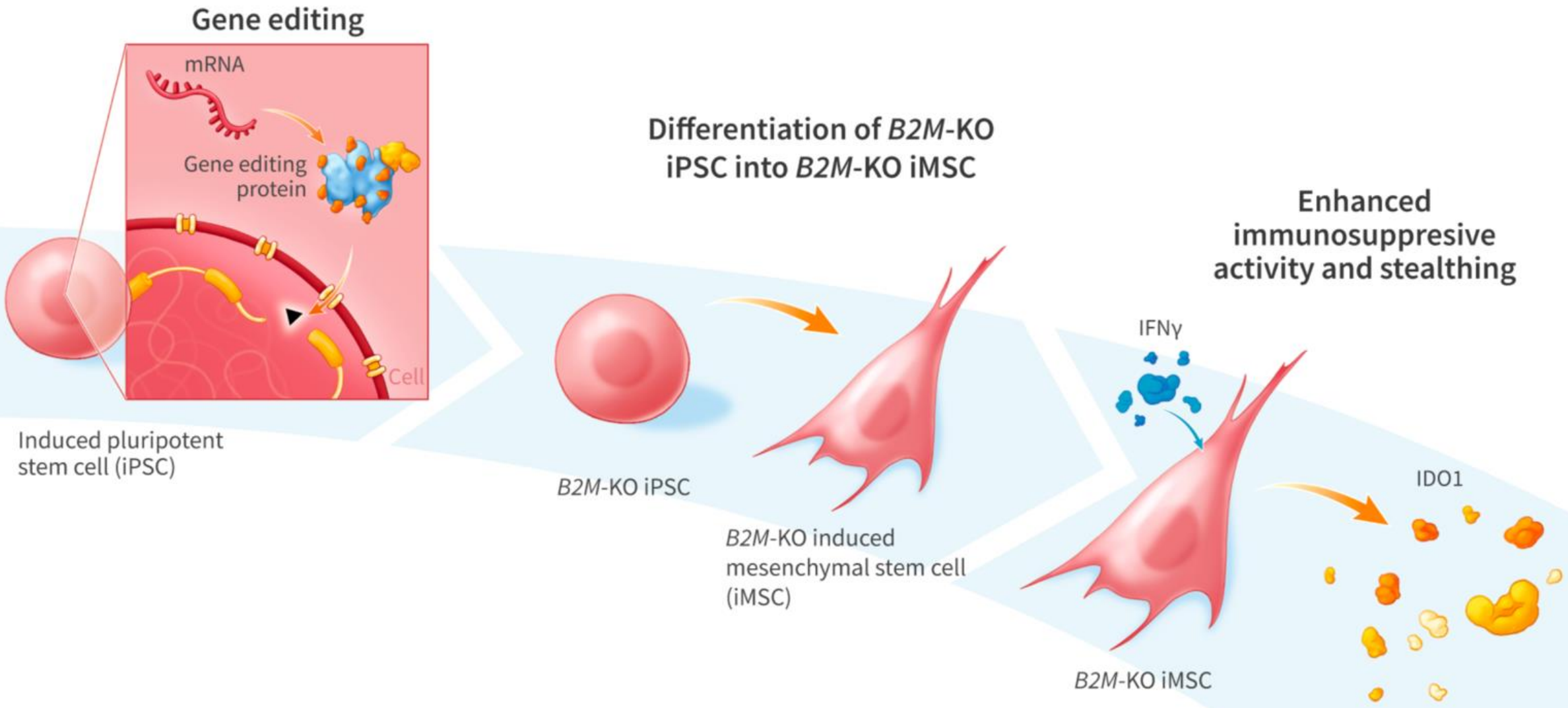
Disclosures

R.H., E.B., K.G., C.R., and M.A. are employees of Factor Bioscience Inc.

C.R. and M.A. are inventors on patents assigned to Factor Bioscience Inc.

B2M-KO iMSCs Exhibit Immunosuppressive Activity & Stealthing Features



MSCs Have Broad Clinical Relevance

Disease	Infusion Method	Cell Source	Study Phase	Serious Adverse Event	NCT Number
Amyotrophic Lateral Sclerosis	I.T	BM-MSC	I	No	NCT01363401
Parkinson's Disease	I.A	BM-MSC	I	No	NCT01824121
Spinal Cord Injury	Subarachnoid	BM-MSC	II	No	NCT0216590
Stroke	I.V	BM-MSC	I	No	NCT01297413
Primary Biliary Cirrhosis	I.V	UC-MSC	N/A	No	NCT01662973
Ischemic-Type Biliary Lesions	I.V	UC-MSC	I	No	NCT02223897
Acute-on-Chronic Liver Failure	I.V	BM-MSC	N/A	No	NCT01322906
Arrhythmogenic Right Ventricular Dysplasia	I.A	AD-MSC	Ia	No	NCT02266394
Chronic Kidney Disease	I.V	BM-MSC	I	No	NCT02195323
Heart Failure	I.V	UC-MSC	I/II	No	NCT01739777
Refractory Angina	Intramyocardial	AD-MSC	N/A	No	NCT01449032
Osteoarthritis	Intra-Articular	BM-MSC	I/II	No	NCT02351011
Bone Fracture	Percutaneous	BM-MSC	I/II	No	NCT02020590
Diabetic Foot Ulcers	N/A	AD-MSC	N/A	No	NCT02619877
Uterine Injury	Intrauterine	UC-MSC	I	No	NCT03386708
Vocal Fold	Local injection	BM-MSC	I/II	No	NCT01981330

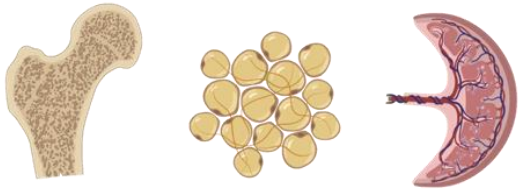
** Adapted From: Margiana, R., Markov, A., Zekiy, A.O. et al. Clinical application of mesenchymal stem cell in regenerative medicine: a narrative review. Stem Cell Res Ther **13**, 366 (2022)*

iPSC Derived MSCs Address the Shortcomings of Tissue Derived MSCs

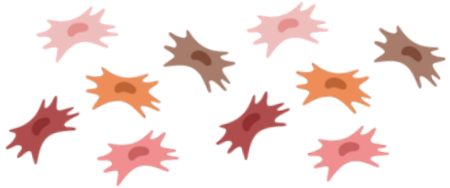
Tissue-Derived MSCs

Donor Variability

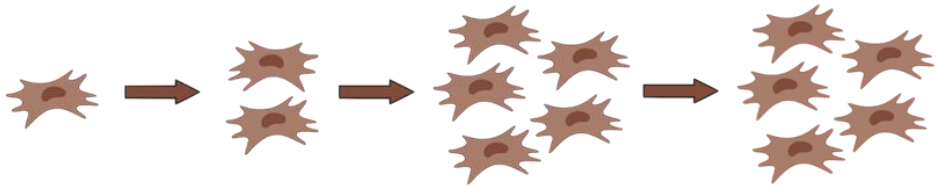
Tissue Source Variability



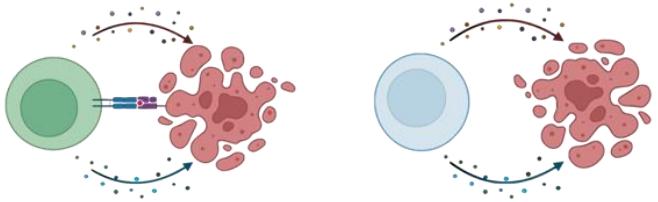
Cellular Heterogeneity



Limited Cellular Expansion Potential



Premature Clearance via Host Immune Cells



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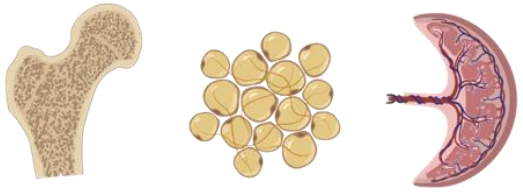
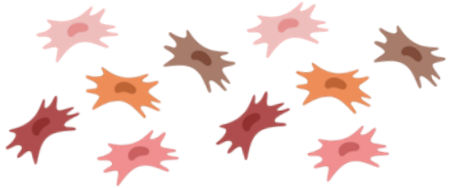
Tissue-Derived MSCs	iPSC Derived MSCs

Donor Variability

Tissue Source Variability



Cellular Heterogeneity



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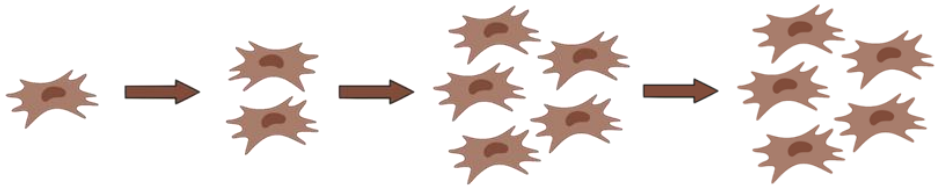

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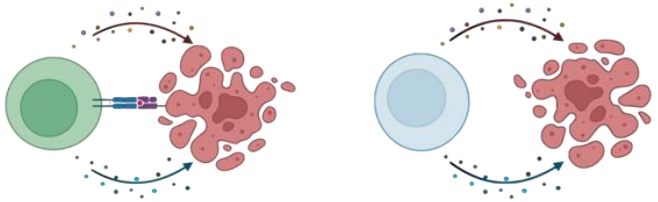
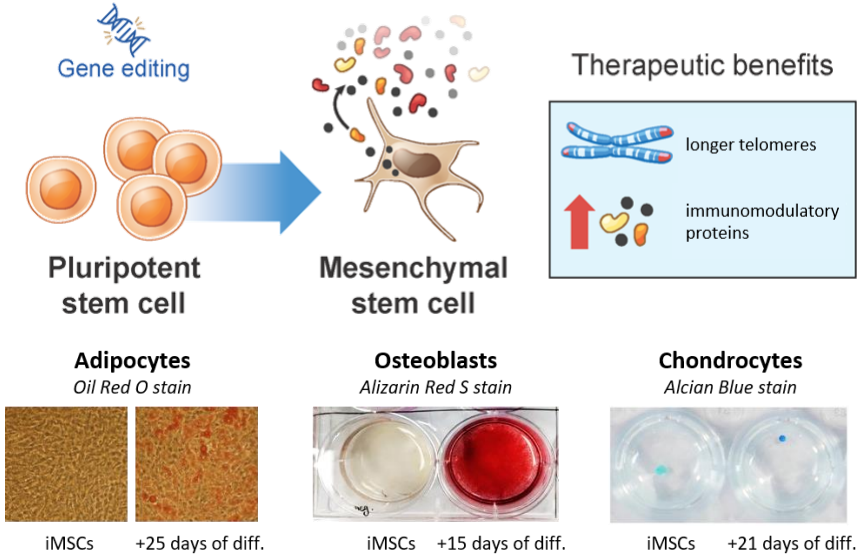
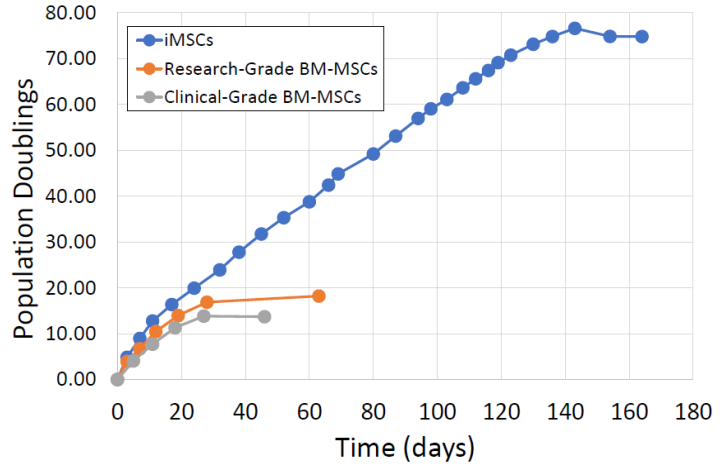






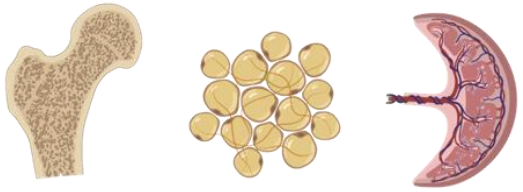
Harris, J. et. al., Mol Ther Vol 28, 2020

iPSC Derived MSCs Address the Shortcomings of Tissue Derived MSCs

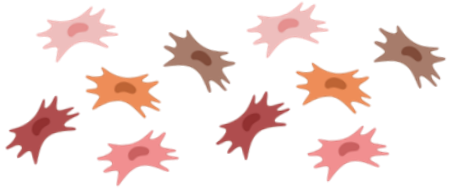
Tissue-Derived MSCs	iPSC Derived MSCs

Donor Variability

Tissue Source Variability



Cellular Heterogeneity



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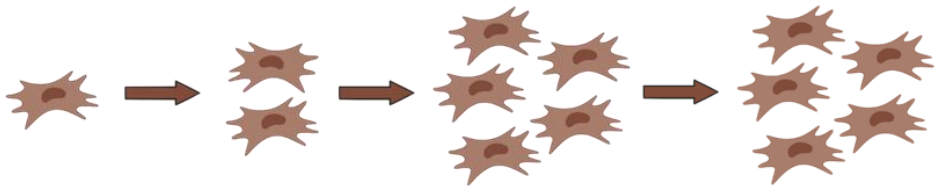
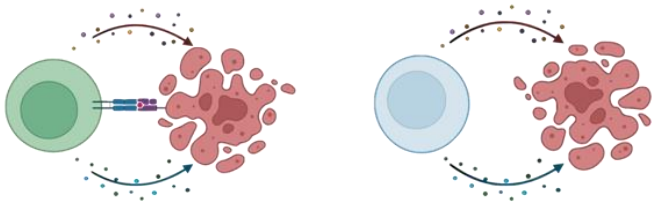
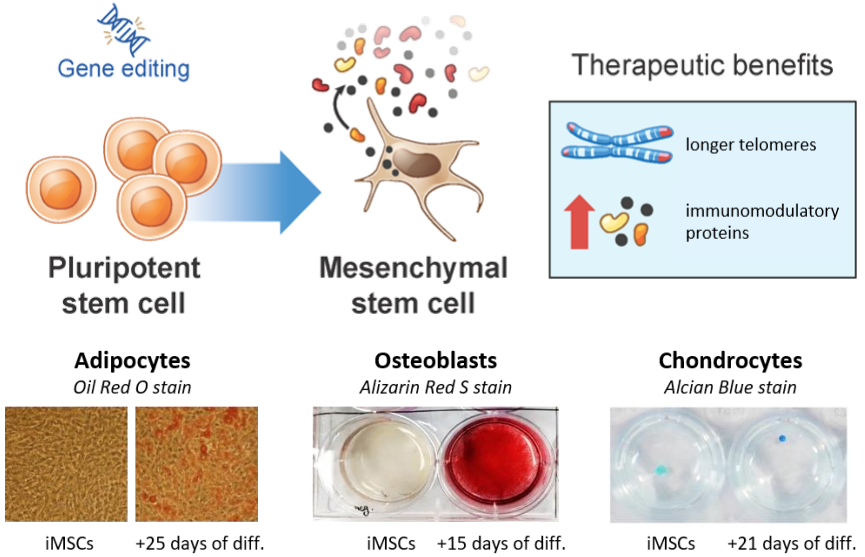
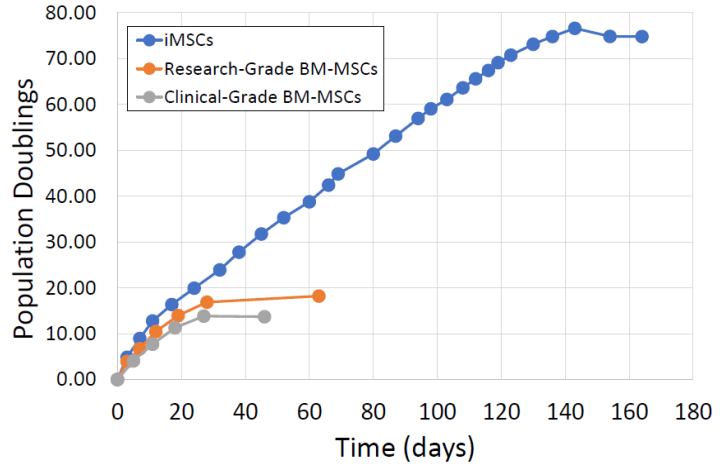


Premature Clearance via Host Immune Cells

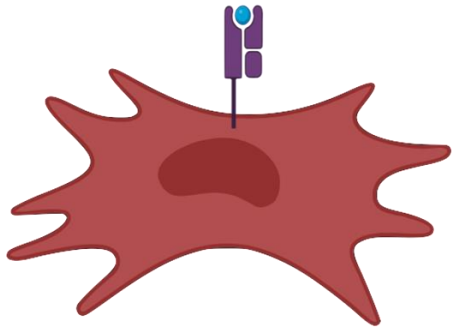






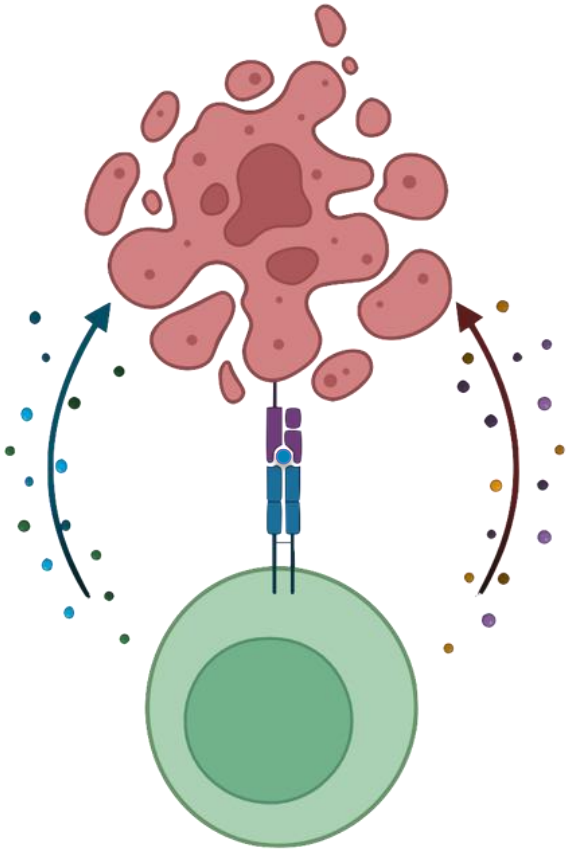
Harris, J. et. al., Mol Ther Vol 28, 2020

Knockout of B2M Address Premature Immune Clearance of MSCs



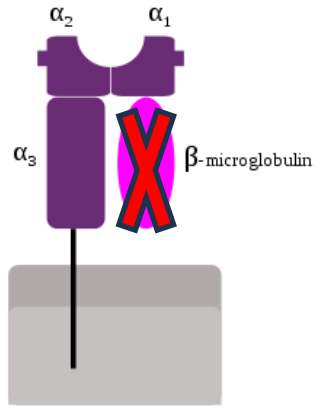
iMSC Expressing HLA-I

T Cell Mediated
Immune Clearance



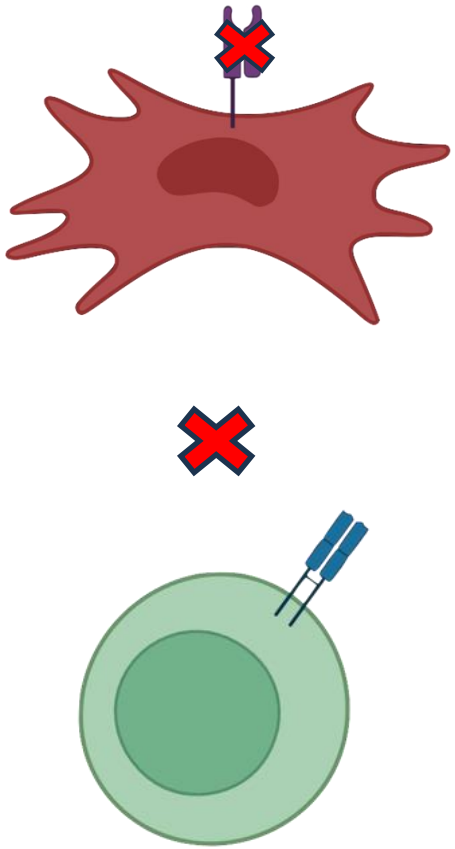
Cytotoxic CD8+ T Cell

HLA-I

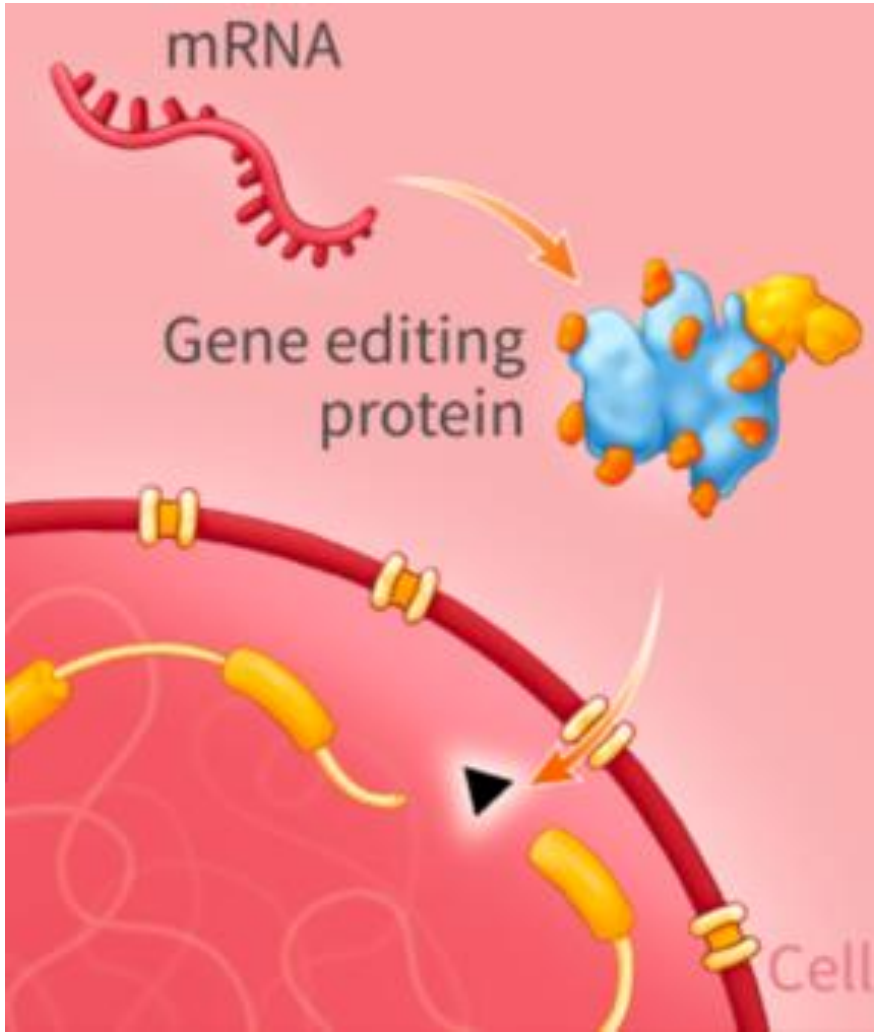


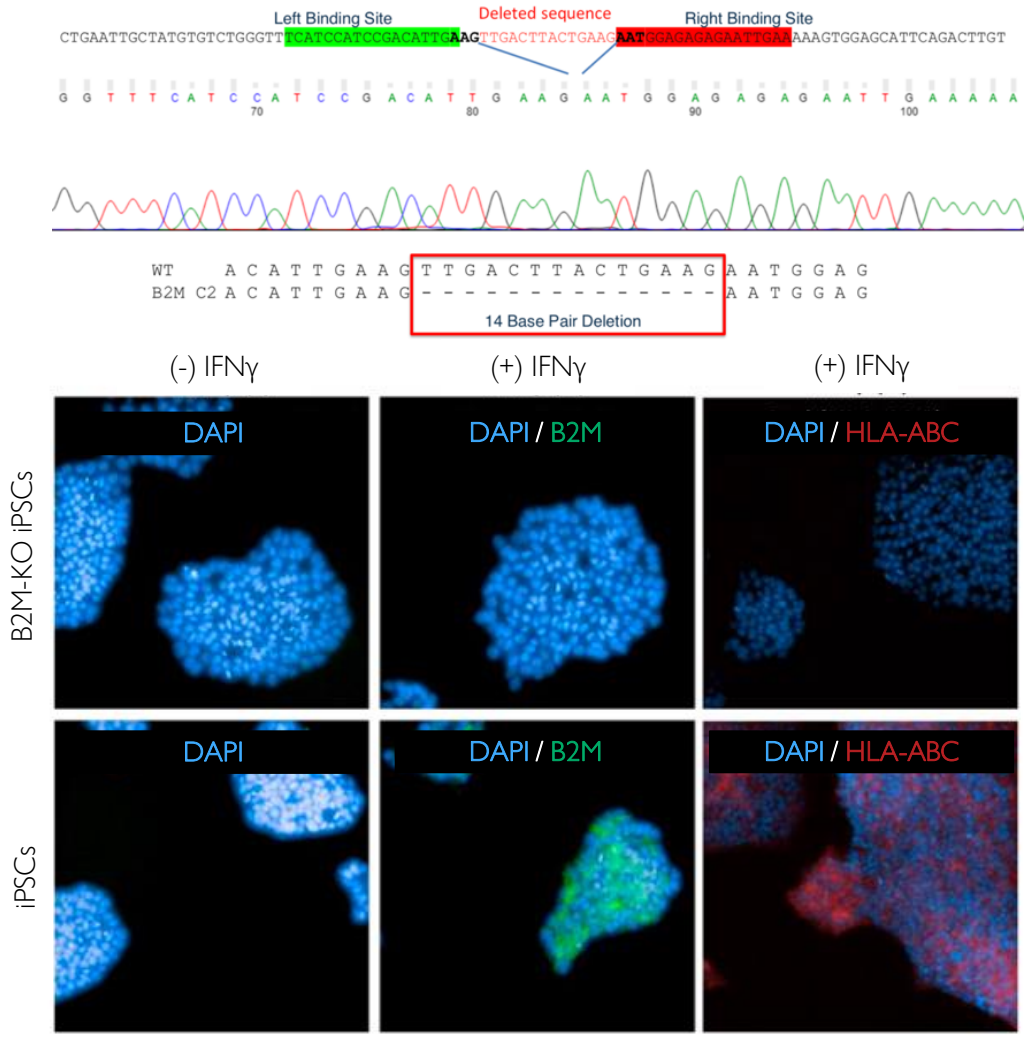
KO of B2M

Stealthing from
T Cell Recognition



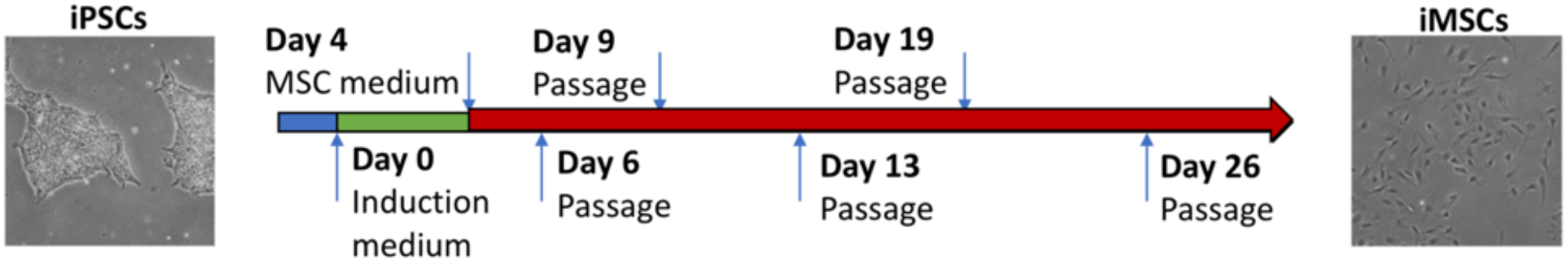
Generation of B2M-KO iPSCs





Kopacz, M. et al., Mol Ther Vol 29, 2021

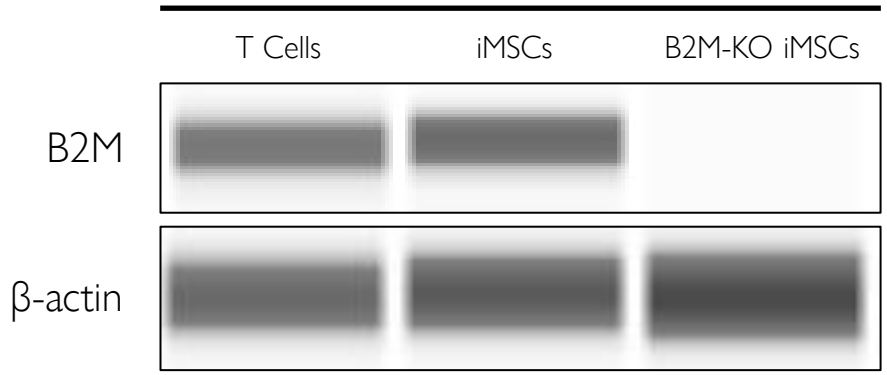
Differentiation of B2M-KO iPSCs → iMSCs

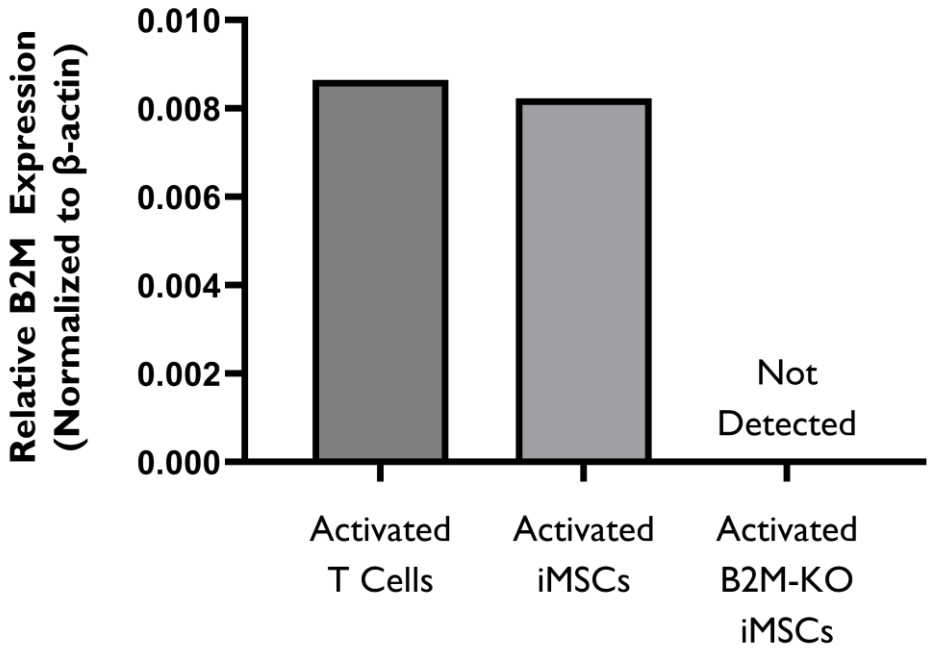


	ISCT-Defined MSC Markers			iPSC Marker	HSC Marker
	CD73	**CD105**	**CD90**	**TRA-1-81**	**CD34**
B2M-KO iMSCs	99%	99%	99%	<1%	<1%
Control Cells	<1% *iPSCs*	<1% *iPSCs*	<5% *PBMCs*	99% *iPSCs*	90% *iPSC-Derived HSCs*

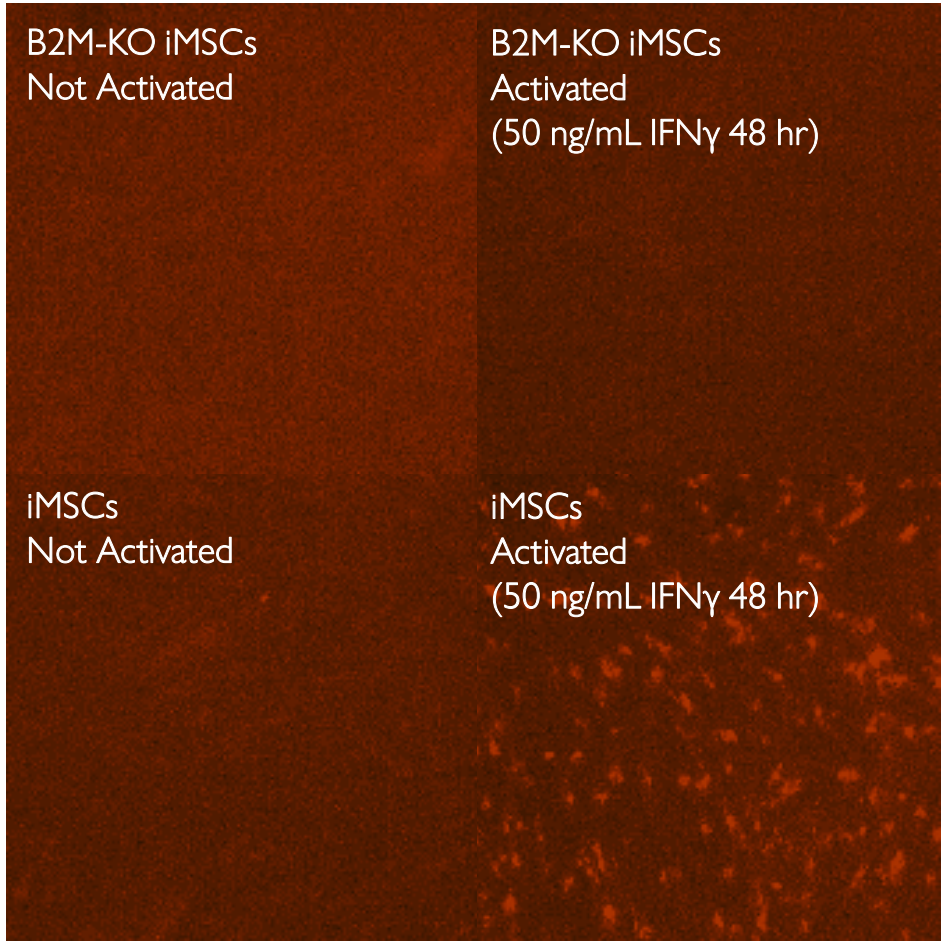
B2M-KO iMSCs Do Not Express B2M

+50 ng/mL IFNγ, 48hr



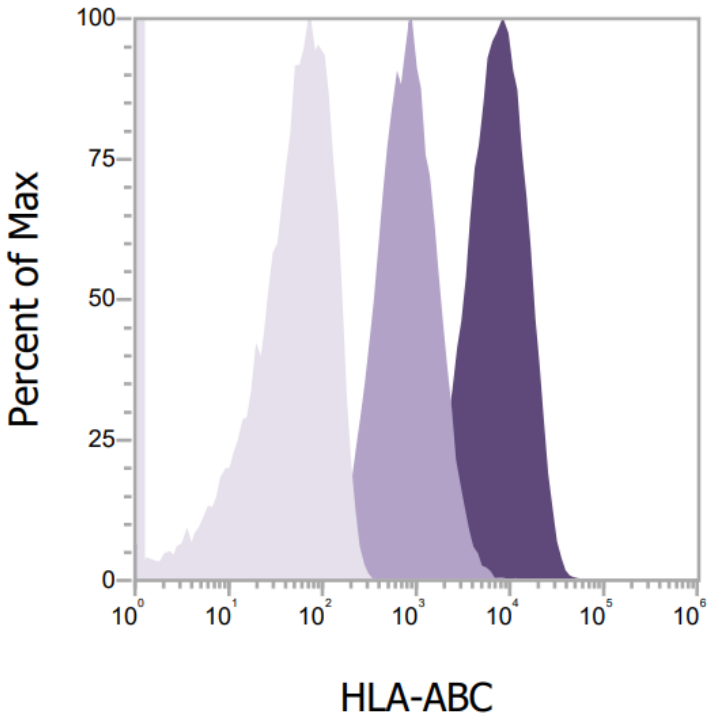


B2M Immunofluorescence



B2M-KO iMSCs Do Not Express HLA-I

iMSCs

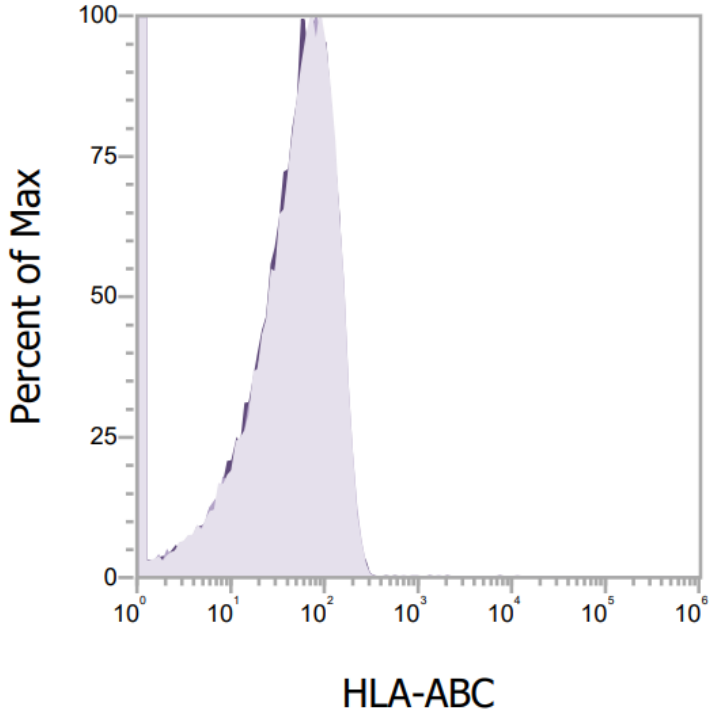


(-) HLA-ABC Stain

iMSCs

iMSCs + IFNγ

BM-KO iMSCs



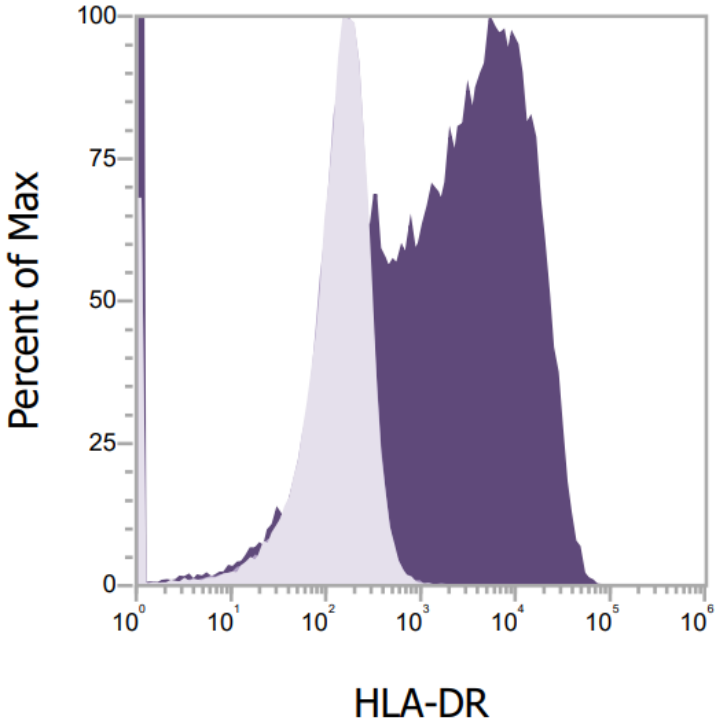
(-) HLA-ABC Stain

B2M-KO iMSCs

B2M-KO iMSCs + IFNγ

B2M-KO iMSCs Do Not Express HLA-DR

iMSCs

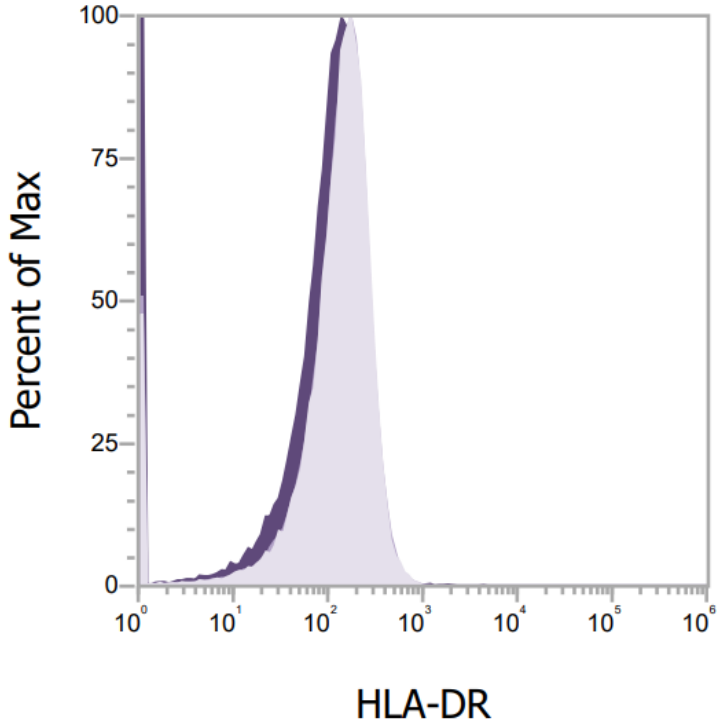


(-) HLA-DR Stain

iMSCs

iMSCs + IFNγ

BM-KO iMSCs

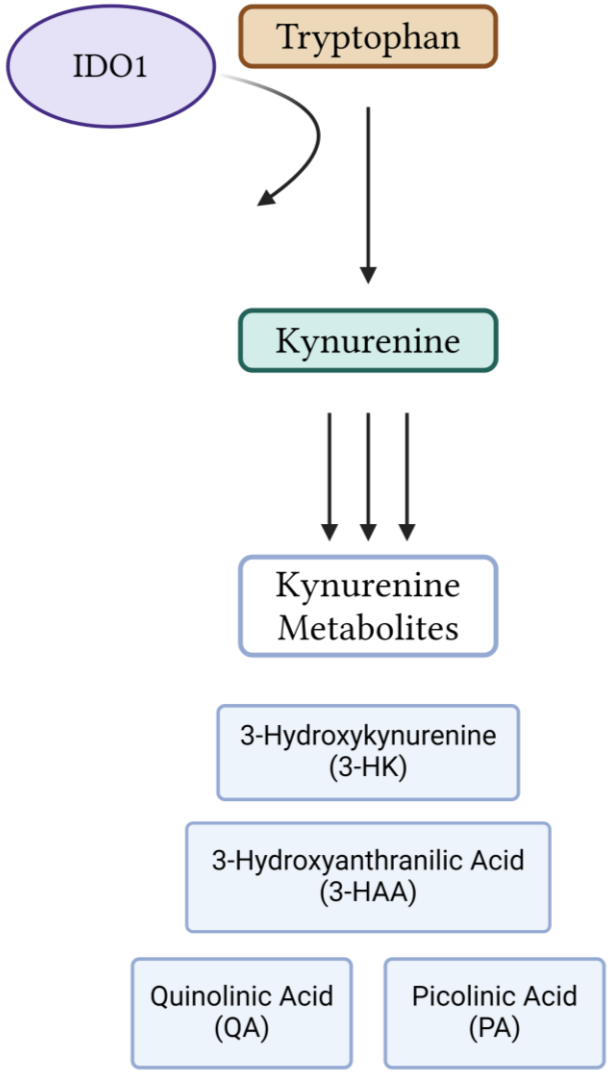


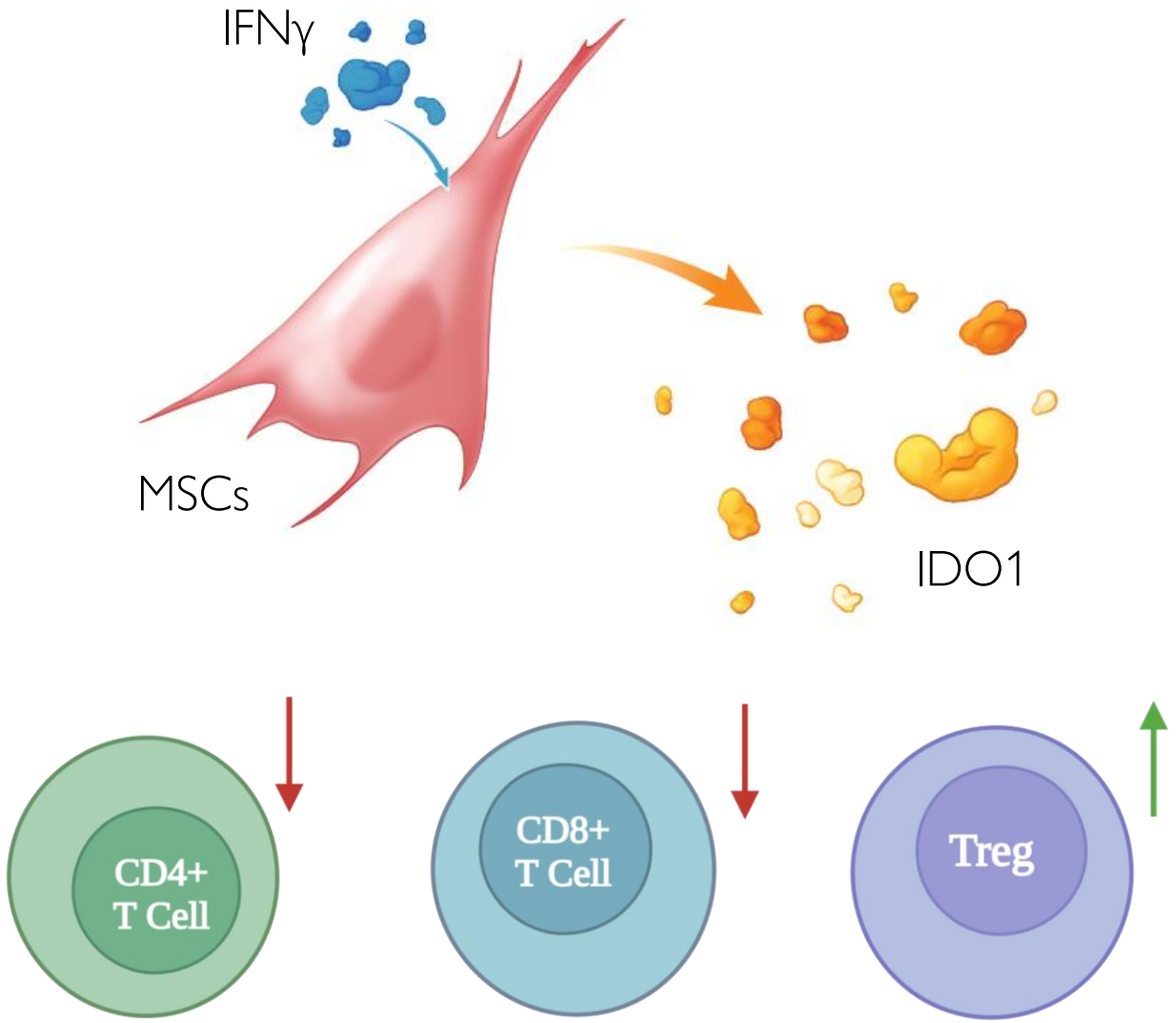
(-) HLA-DR Stain

B2M-KO iMSCs

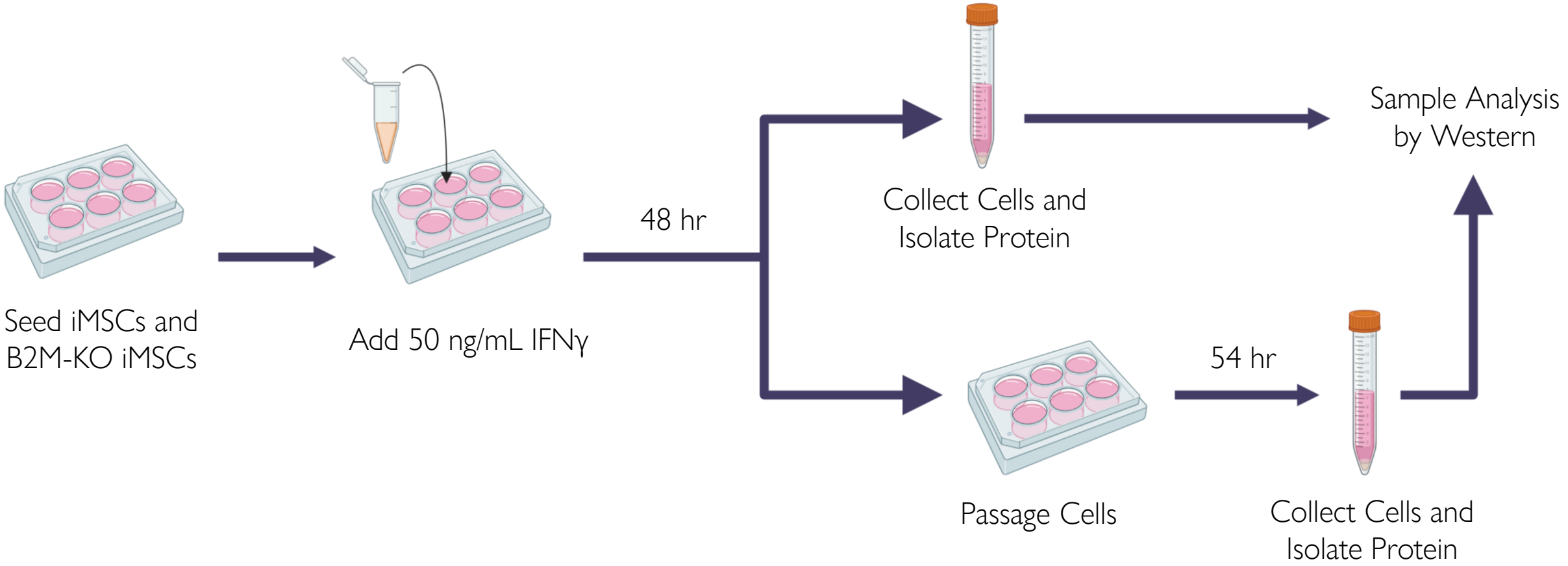
B2M-KO iMSCs + IFNγ

IDO1 is A Key Modulator of MSC Immunosuppression

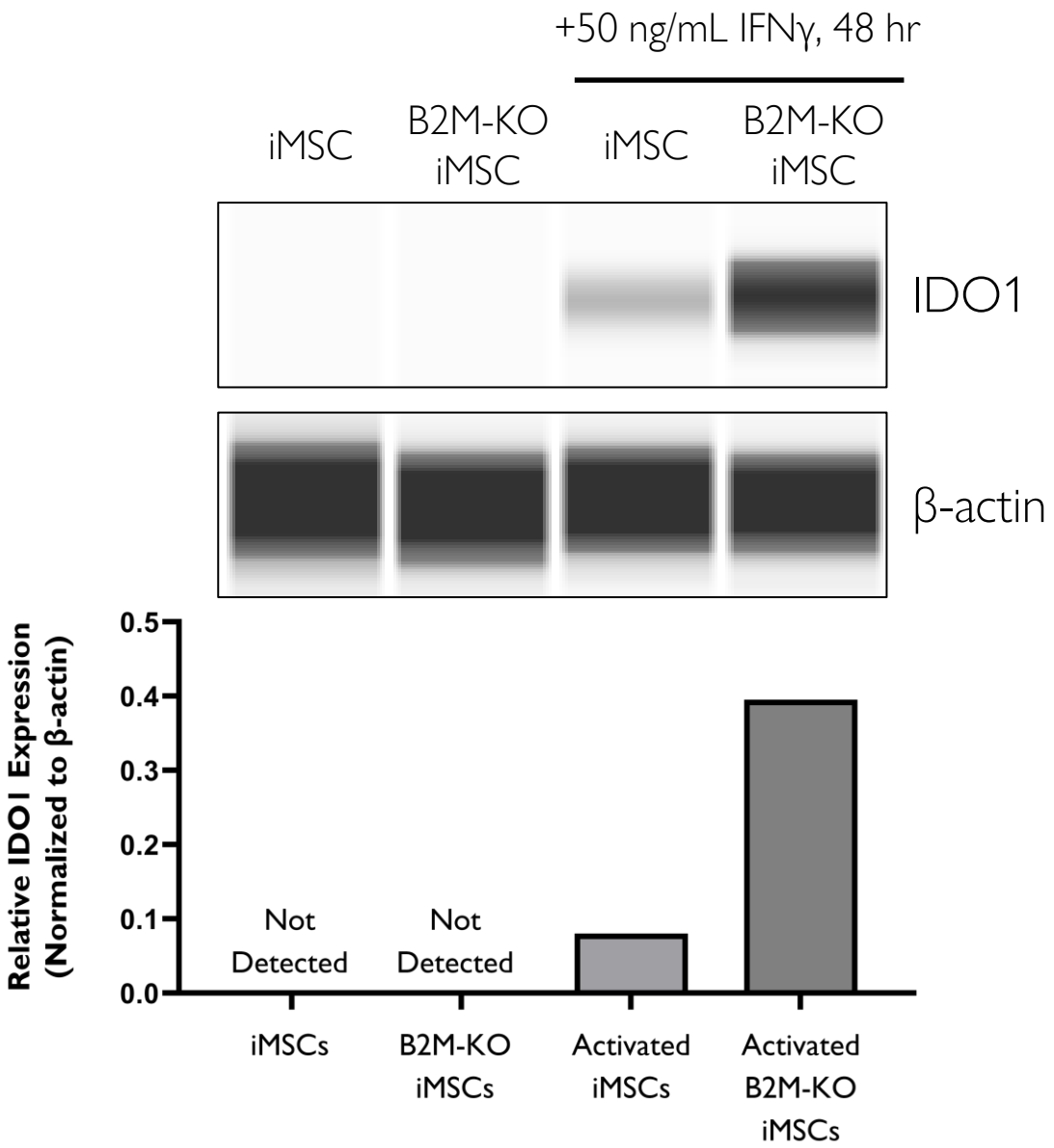


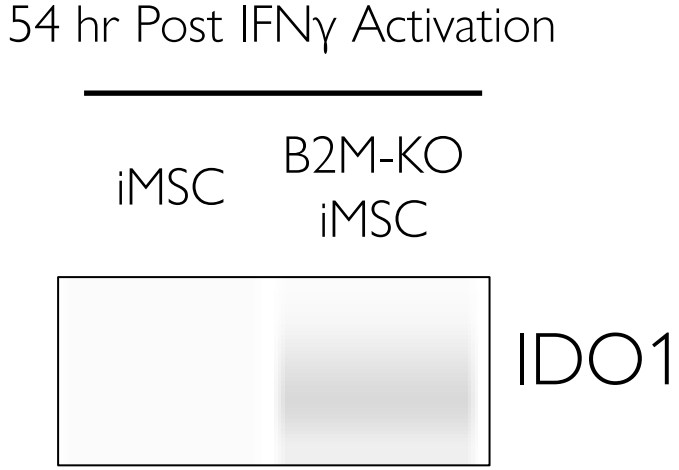


Assay Setup: IDO1 Western

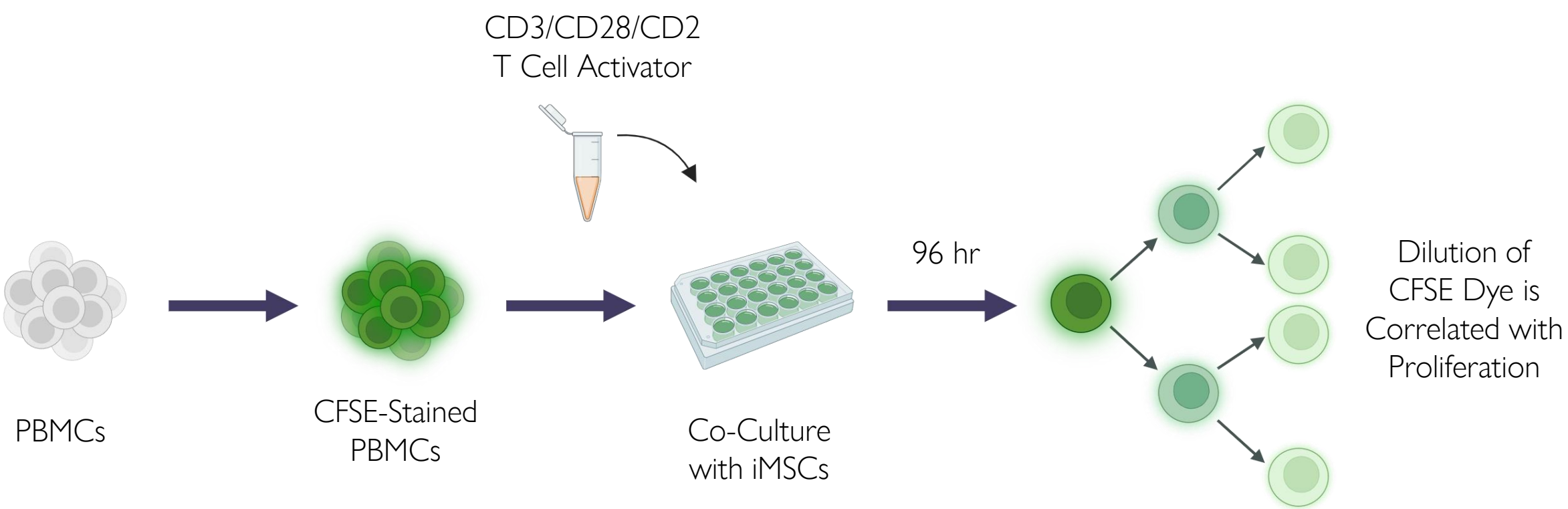


B2M-KO iMSCs Express More IDO1 than Native iMSCs

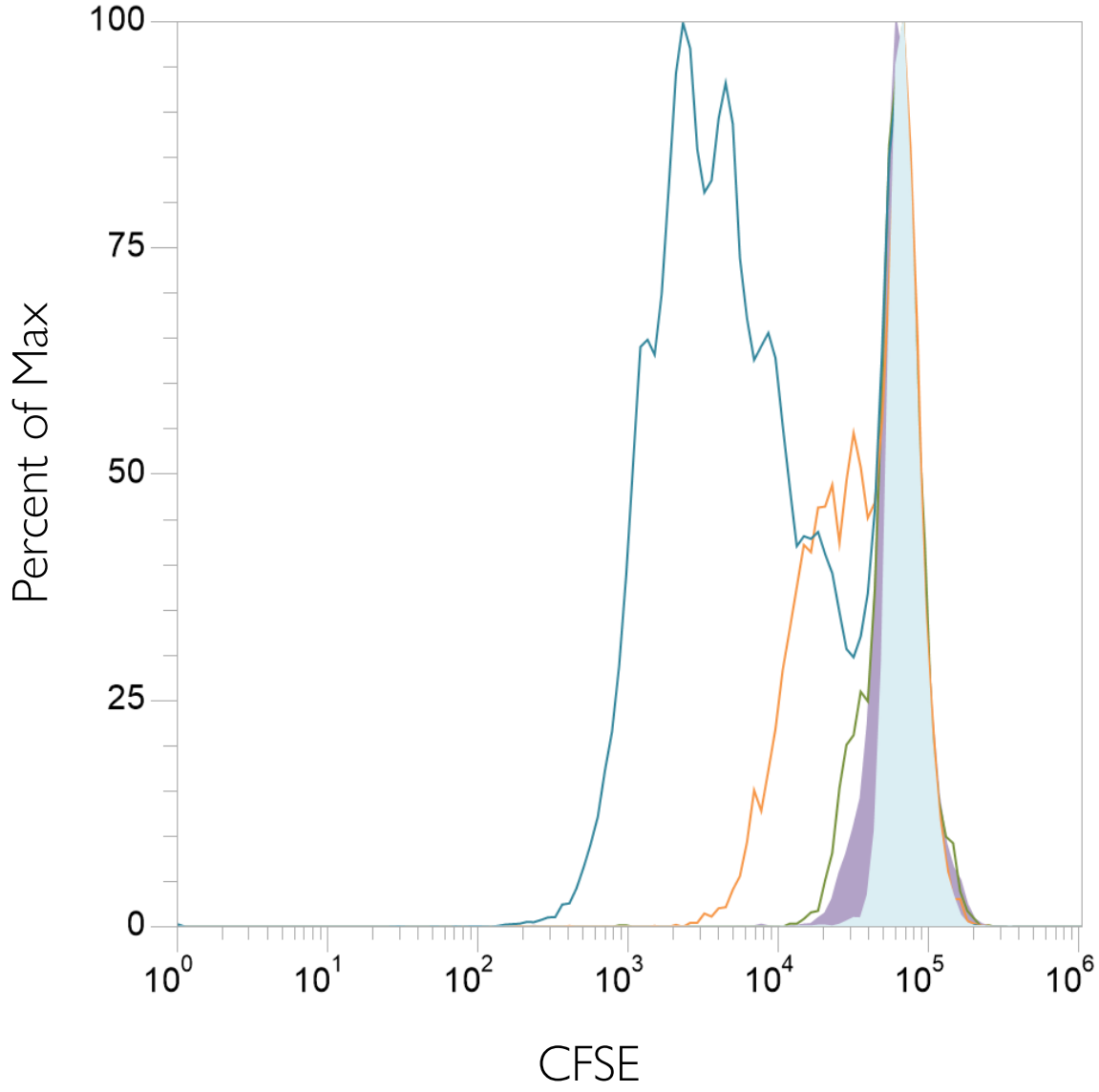




Assay Step Up: PBMC Suppression



B2M-KO iMSCs Better Suppress PBMC Proliferation



PBMCs

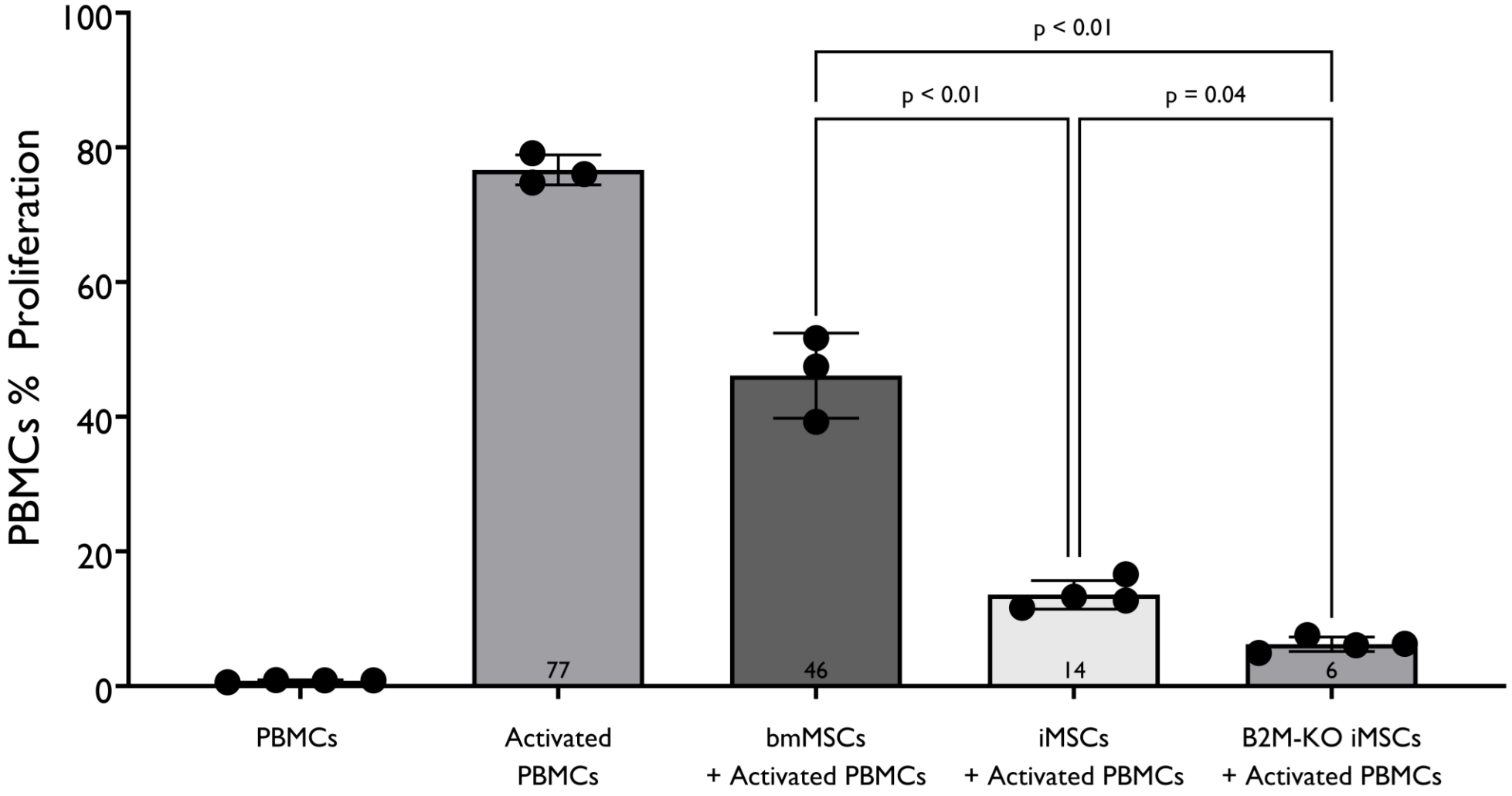
Activated PBMCs

Activated PBMCs + bmMSCs

Activated PBMCs + iMSCs

Activated PBMCs + B2M-KO iMSCs

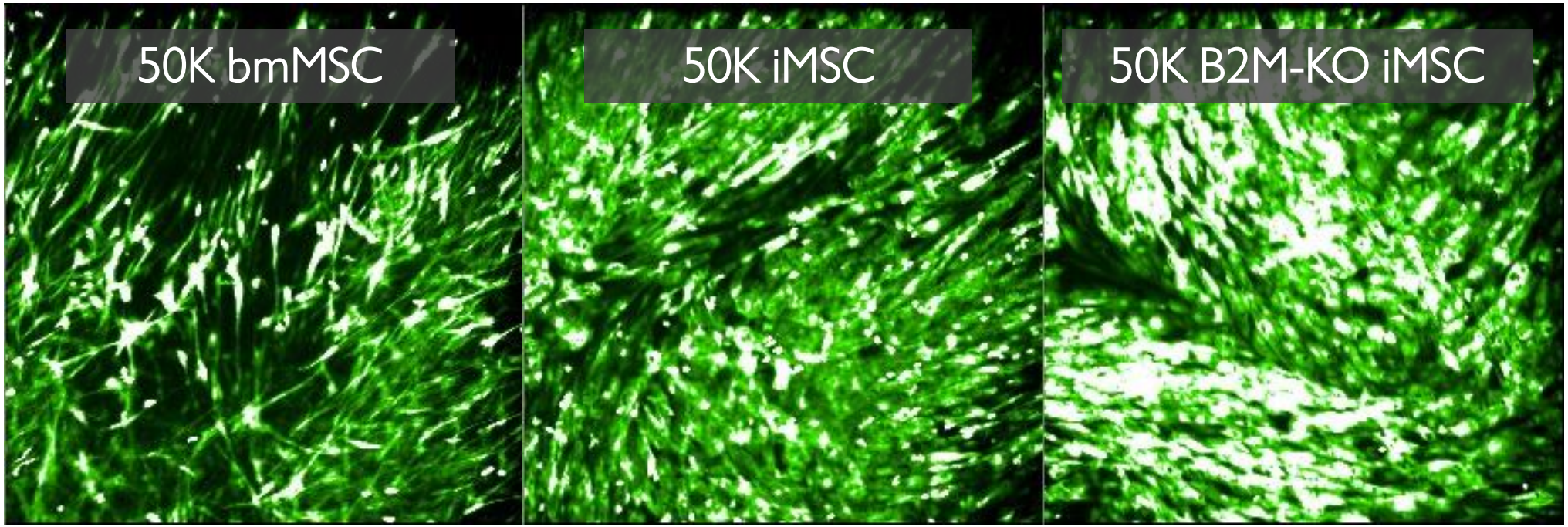
B2M-KO iMSCs Better Suppress PBMC Proliferation



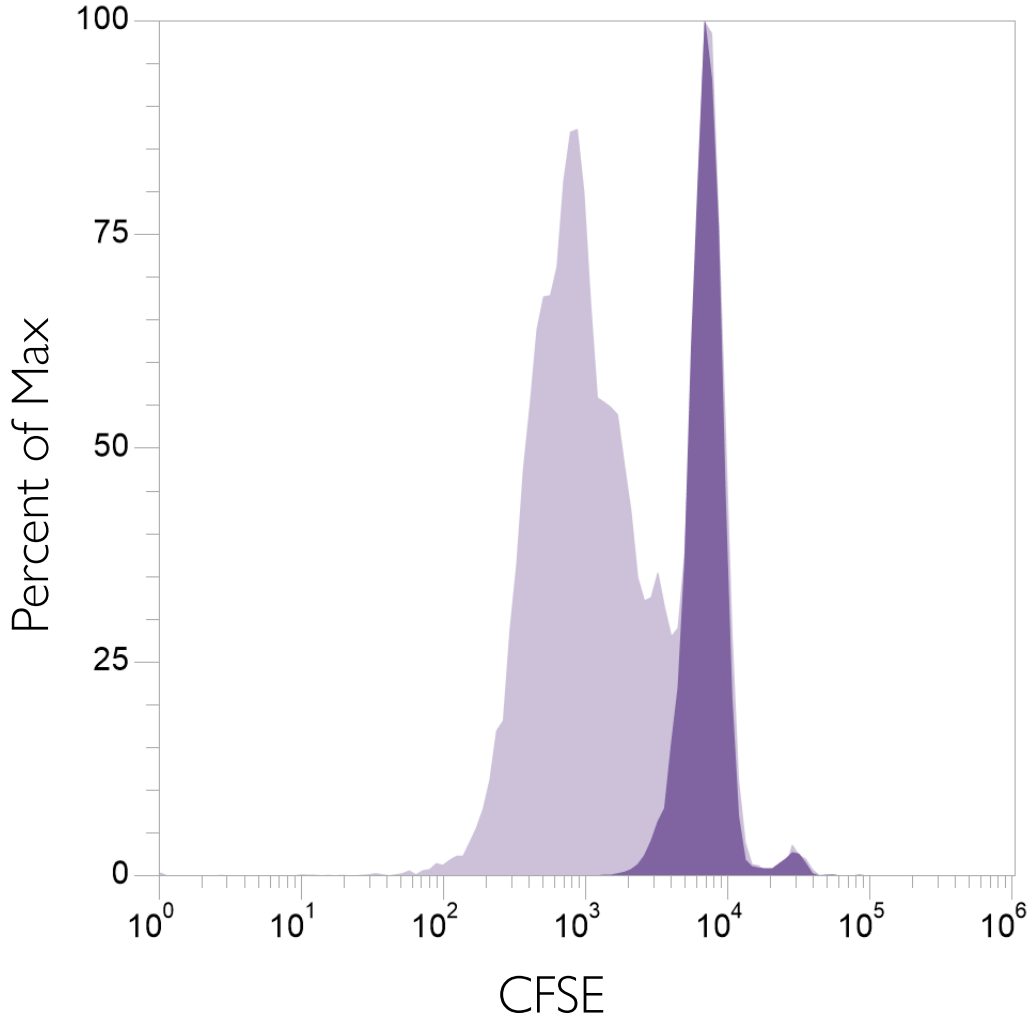
B2M-KO iMSCs Better Suppress PBMC Proliferation

+Activated PBMCs, 4 days

IDO1 (AF488)



IDO1 is Critical for the Suppression of PBMCs



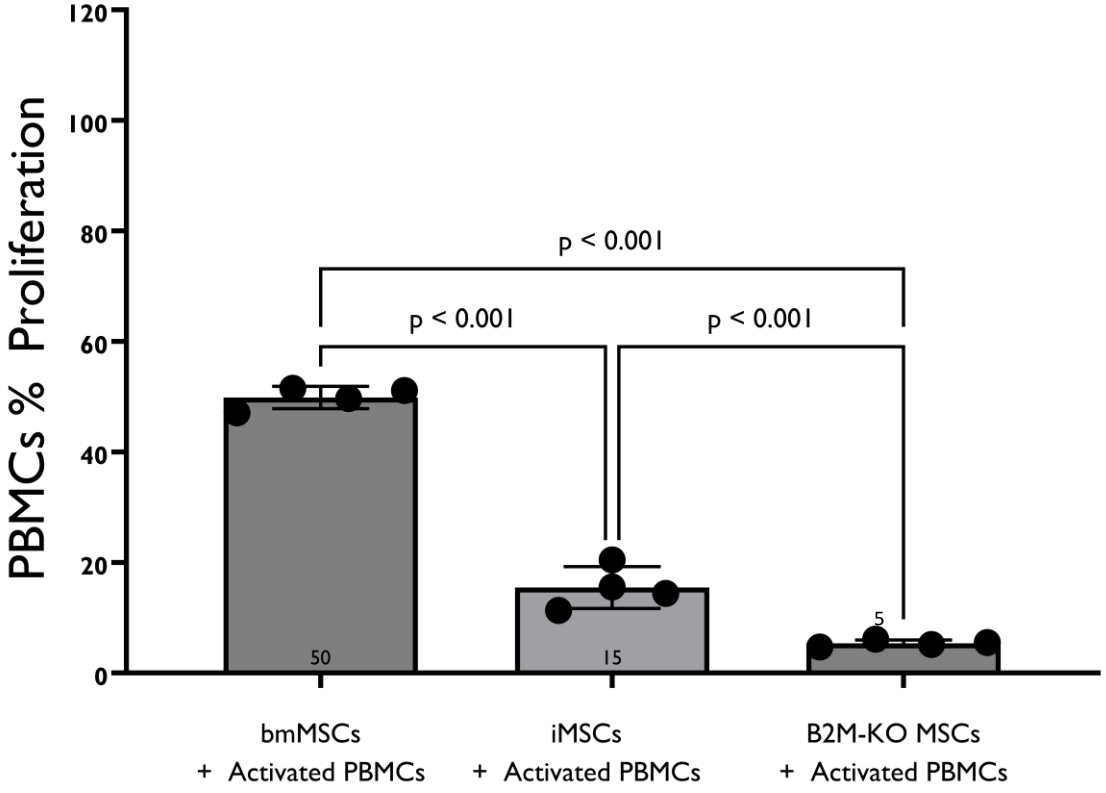
Activated PBMCs + B2M-KO iMSCs

Activated PBMCs + B2M-KO iMSCs + IDO1 Inhibitor

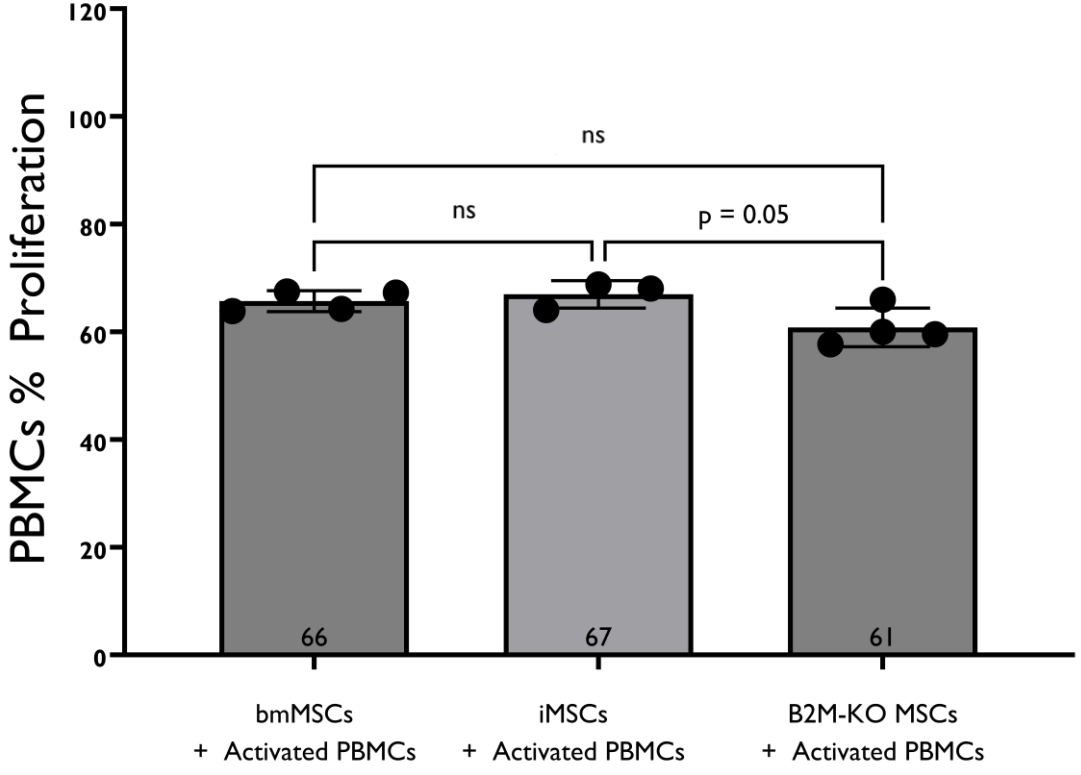
IDO1 is Critical for the Suppression of PBMCs

PBMCs

Without IDO1 Inhibitor



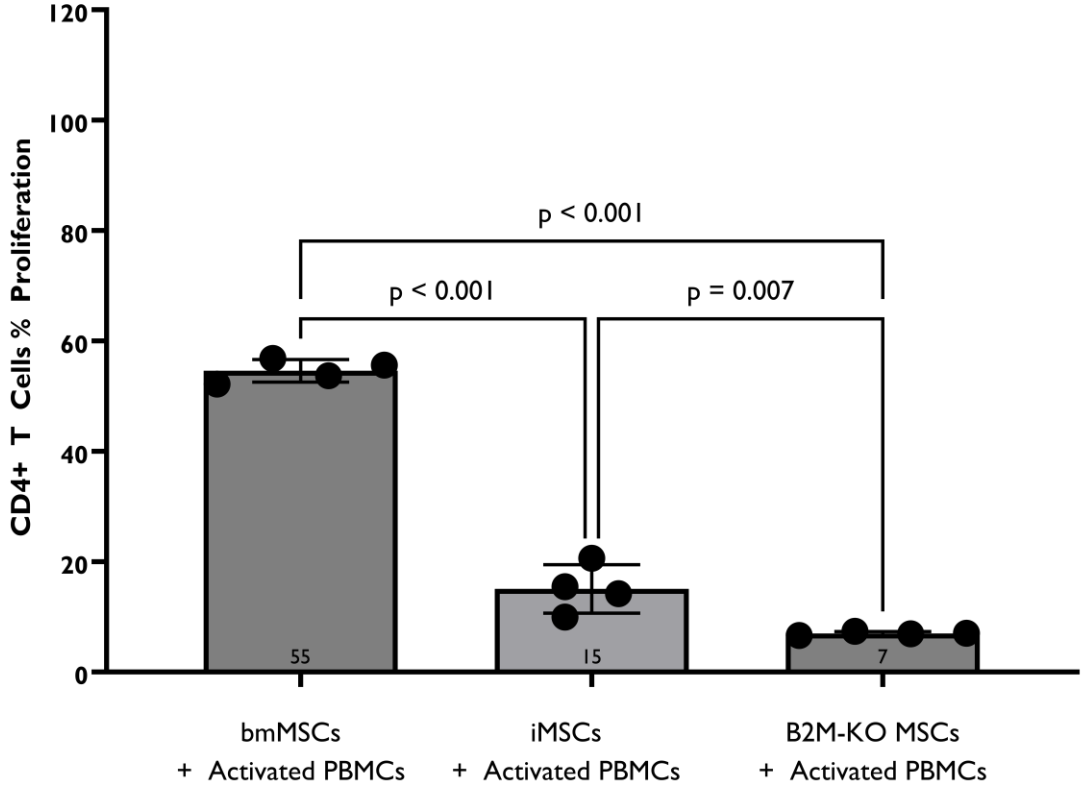
With IDO1 Inhibitor



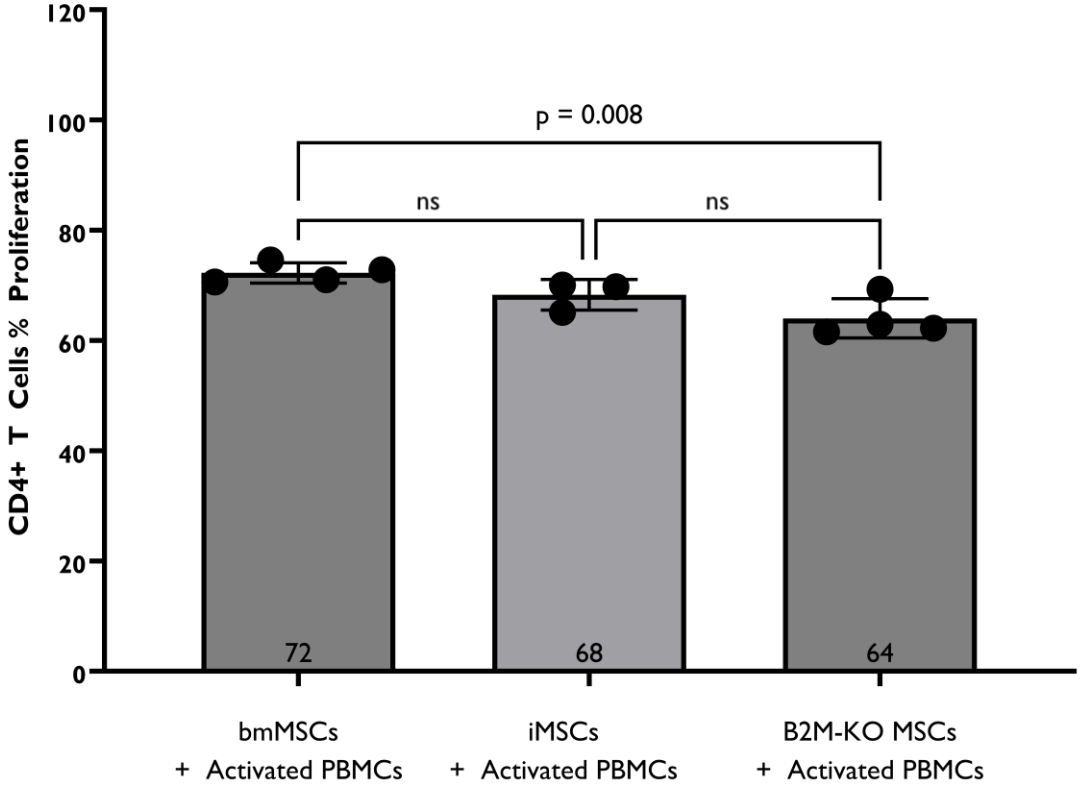
IDO1 is Critical for the Suppression of CD4+ T Cells

CD4+ T Cells

Without IDO1 Inhibitor



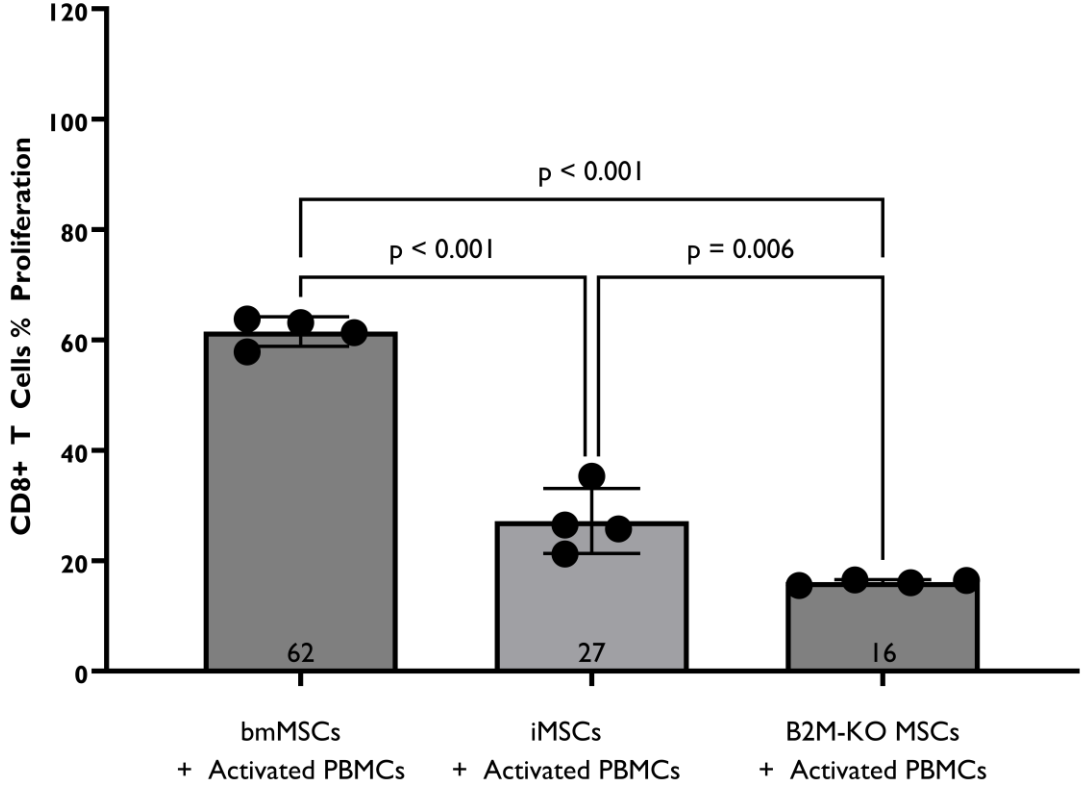
With IDO1 Inhibitor



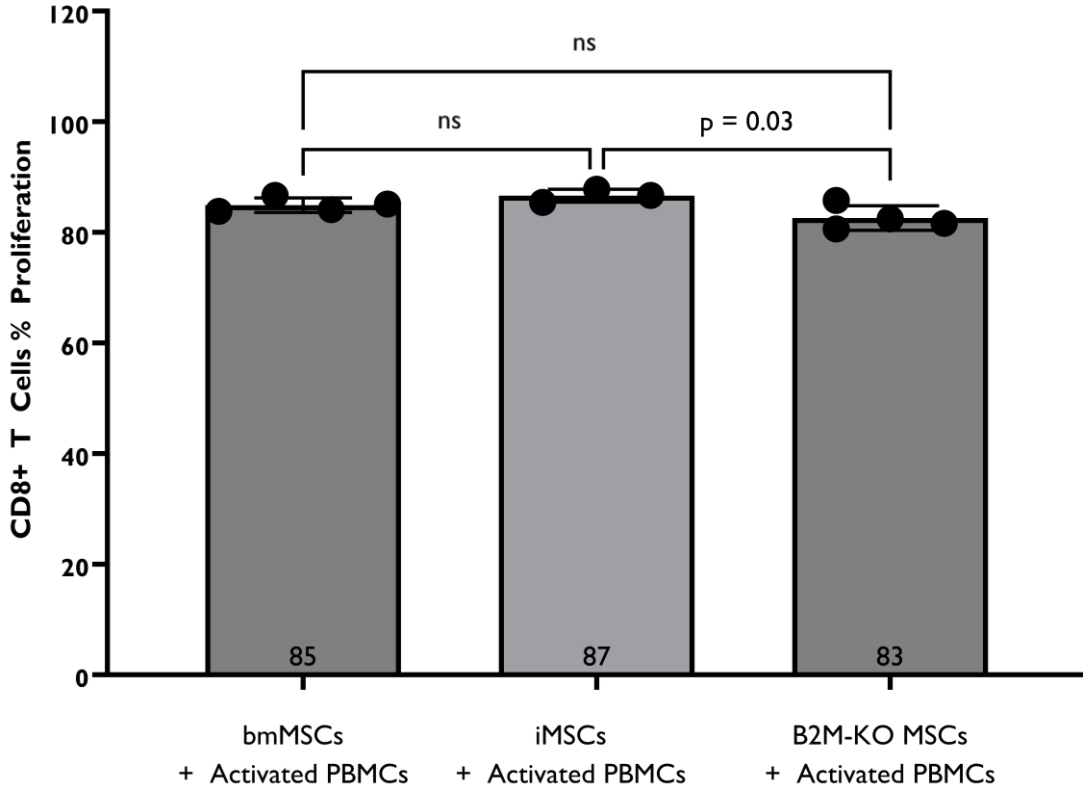
IDO1 is Critical for the Suppression of CD8+ T Cells

CD8+ T Cells

Without IDO1 Inhibitor



With IDO1 Inhibitor

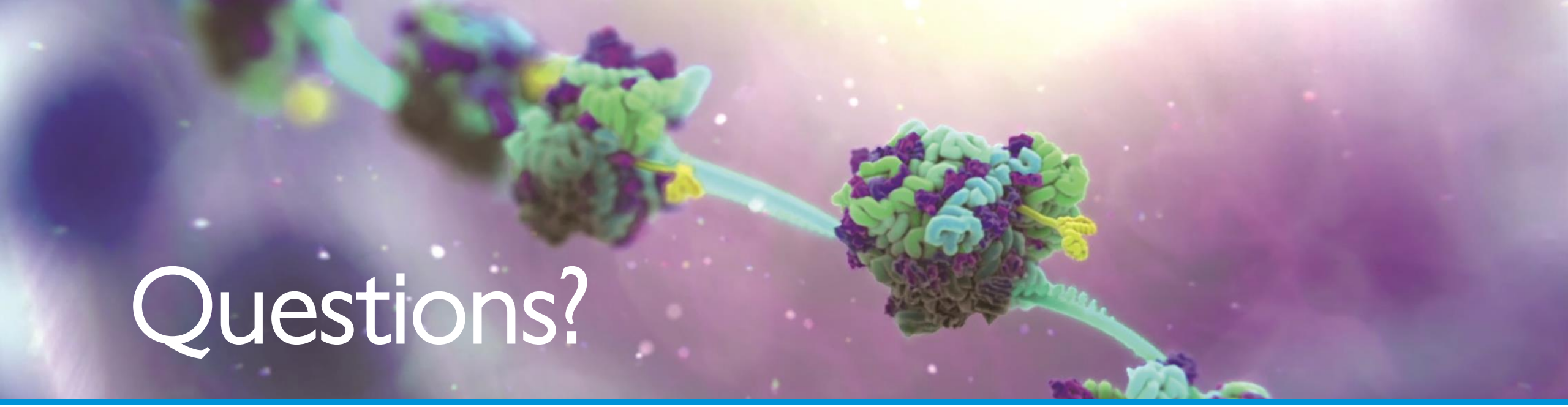


Summary & Looking Forward

B2M-KO iMSCs

- ✓ Do not express HLA-I or the HLA-II molecule, HLA-DR
- ✓ More likely to evade immune clearance by CD8+ T Cells
- ✓ Express higher levels of IDO1 following IFNγ stimulation
- ✓ Improved ability to inhibit CD4+ and CD8+ T Cell proliferation

Acknowledgements



Questions?

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