



Beyond HLA Silencing: Five-Gene TARGATT™ Engineering of Hypoimmune iPSCs

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ABSTRACT

Background

Allogeneic approaches represent a promising strategy for the drug discovery industry to develop scalable and cost-effective cell therapies, but immune rejection of donor-derived cells remains an ongoing challenge [1]. Applied StemCell combines advanced iPSC genome editing with patented TARGATT™ large-cargo gene knock-in technology to develop the Hypoimmunogenic Plus cell line platform and accelerate next-generation cell therapies. AST-9750 integrates B2M and CIITA knockout with TARGATT™-mediated insertion of a five-gene immune-evasion cassette encoding HLA-F-B2M, CD200, CCL21, HLA-G-B2M, and SERPINB9. This multiplex genome-engineered human iPSC platform is designed to reduce T-cell, natural killer (NK), and dendritic cell recognition, retain pluripotency, enable therapeutic gene expression, and support scalable manufacturing and development of next-generation allogeneic cell therapies for research, drug discovery, manufacturing, and clinical translation applications.

Methodology

Using MAD7 gene editing technology, we've knocked out the HLA class I and II molecules (B2M/CIITA double knock-out) in our TARGATT™ RUO hiPSCs (AST-9450) to generate TARGATT™ RUO Hypo hiPSCs (AST-9650). The combination of B2M & CIITA knock-out has been previously shown to effectively protect against T-cell mediated immune responses [2]. However, these two knockouts alone are not sufficient to protect against Natural Killer (NK)-, Dendritic-, and Macrophage- mediated immune responses. To address a complete range of expected immune-mediated response, we have knocked-in a 5-transgene cassette (HLA-F-B2M, CD200, CCL21, HLA-G-B2M, SERPINB9) into the TARGATT™ [3] landing pad of TARGATT™ RUO Hypo hiPSCs (AST-9650) to generate our newest offering TARGATT™ Hypo Plus Human iPSC, RUO (AST-9750) [4]. This combination of genetic modifications is expected to provide full immune privilege across the complete spectrum of expected immune responses including T-cell, NK cell and Macrophage mediated immune responses.

Results

We show characterization of the hiPSC B2M/CIITA double knock-out cells (AST-9650), verifying the absence of HLA class I and II molecules. We have knocked in a 17kb cassette using the TARGATT™ landing pad and verified transgene expression of two genes (CD200 and SERPINB9) in AST-9750. We have confirmed the pluripotency and normal karyotype of AST-9750.

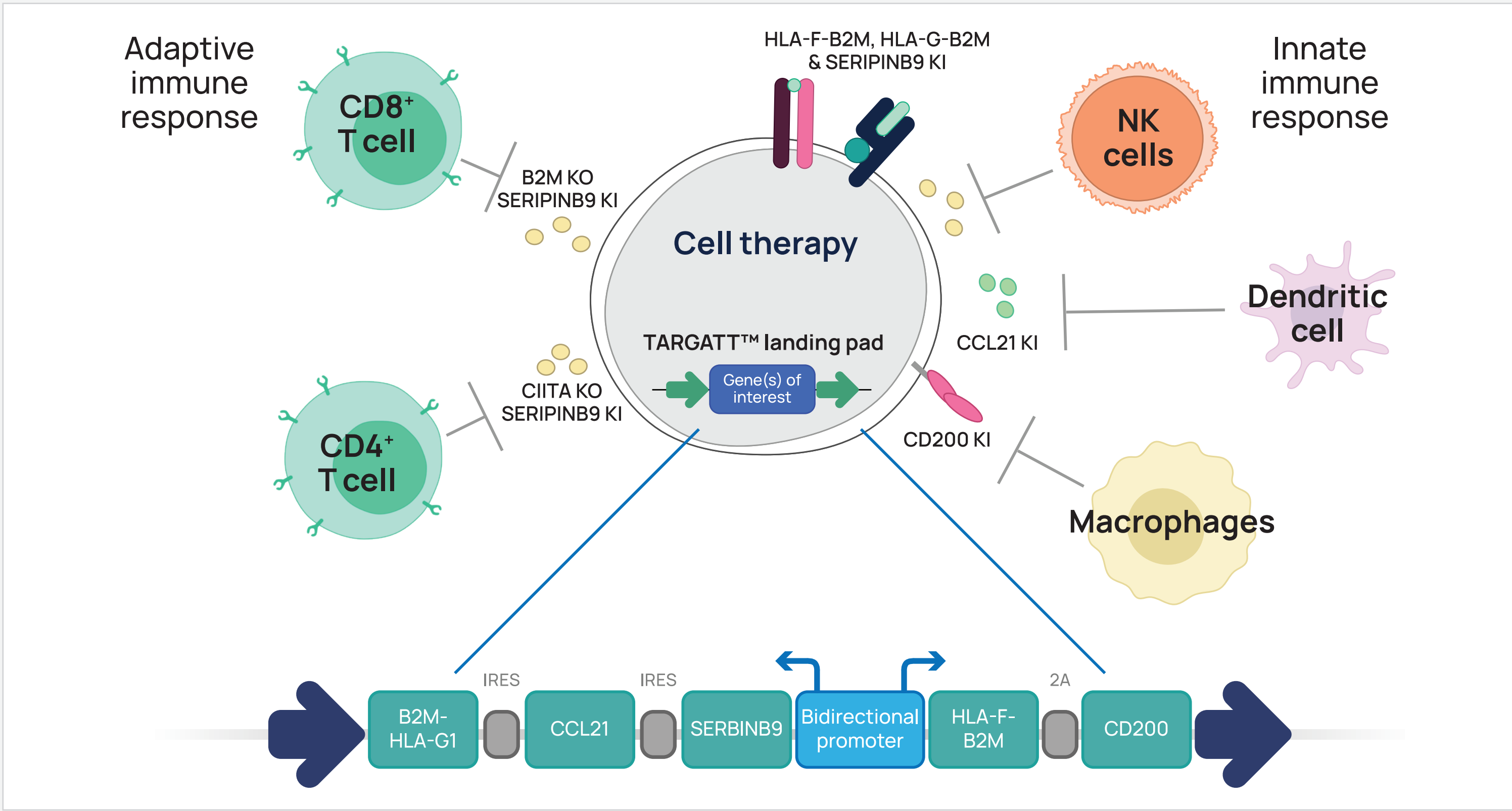
Conclusions

TARGATT™ Hypo Plus Human iPSC, RUO (AST-9750) is a research grade iPSC line which is readily available to jump start your cell therapy applications. The combination of genetic modifications is expected to provide full immune evasion (characterization work is ongoing.) The TARGATT™ Hypo Plus Human iPSC, RUO (AST-9750) are ready for differentiation into your lineage of choice providing a suitable starting material for a wide range of cell therapy applications.

OBJECTIVE & APPROACH

Enable allogeneic cell therapy by developing an iPSC-based cell platform that can persist in the host by evading immune detection and elimination. Implement multiplex-genome engineering to modify expression of seven immune-related genes in iPSCs to enable full immune evasion

Figure 1: TARGATT™ Hypo Plus Human iPSC, RUO (AST-9750) Immune Evasion



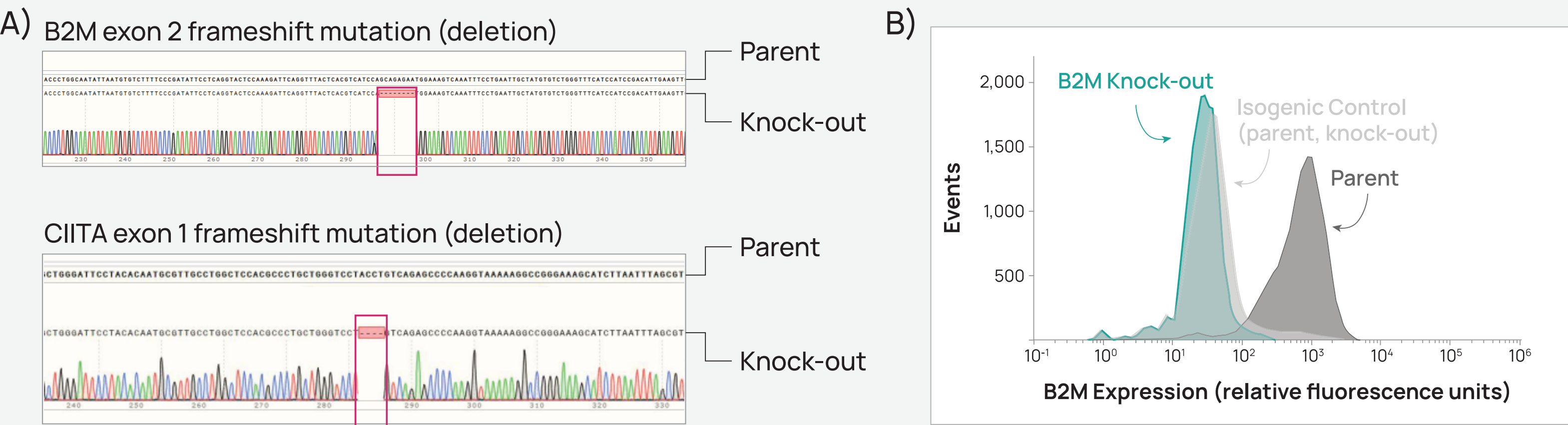
Cell therapy rejection is mediated by a combination of adaptive and innate immune response. TARGATT™ Hypo Plus Human iPSC, RUO (AST-9750) combines B2M and CIITA knockout with TARGATT™-mediated knock-in of a 17 kb/ five-gene immune-evasion construct encoding HLA-F-B2M, CD200, CCL21, HLA-G-B2M, and SERPINB9.

Table 1: Intended Functions of Genetic Modifications

Genetic Modification	Intended Function
B2M Knock-out	Reduce B2M-dependent HLA-I surface expression and direct recognition by alloreactive CD+ T cells; creates missing-self NK-cell risk [5]
CIITA Knock-out	Reduce HLA-II pathway expression and direct recognition by CD4+ T cells [6]
HLA-F-B2M knock-in	Engage inhibitory receptors to reduce missing-self NK-cell cytotoxicity [4]
HLA-G-B2M knock-in	Engage inhibitory receptors to reduce innate immune activation and cytotoxicity [7]
SERPINB9 knock-in	Inhibit cytosolic granzyme B and increase resistance to granzyme-B-mediated apoptosis caused by cytotoxic T and NK cells [8]
CCL21 knock-in	Induce locally tolerogenic immune niche [9]
CD200 knock-in	Suppress macrophage and other myeloid-cell activation, thereby reducing inflammatory and phagocytic clearance [10]

RESULTS

Figure 2: Knock-out of B2M and CIITA Are Experimentally Verified



Enable allogeneic cell therapy by developing an iPSC-based cell platform that can persist in the host by evading immune detection and elimination. Implement multiplex-genome engineering to modify expression of seven immune-related genes in iPSCs to enable full immune evasion

Figure 3: HLA-G and HLA-F Over Expression Reduce NK-mediate Cytotoxicity

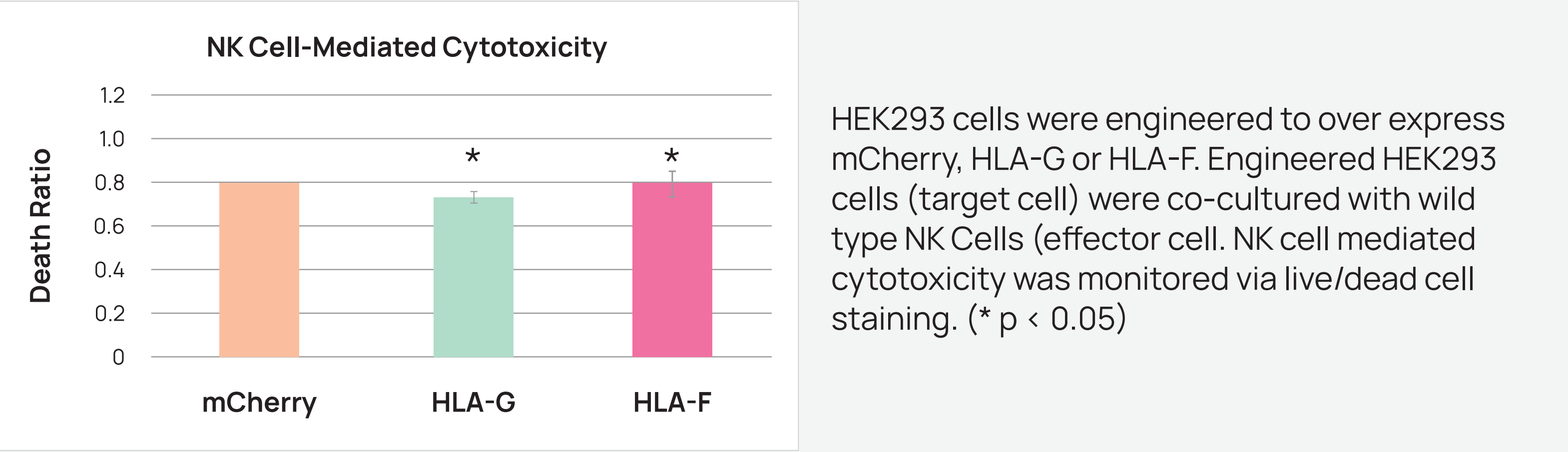


Figure 4: Confirmed Expression of 17kb Hypoimmune Plus Expression Cassette

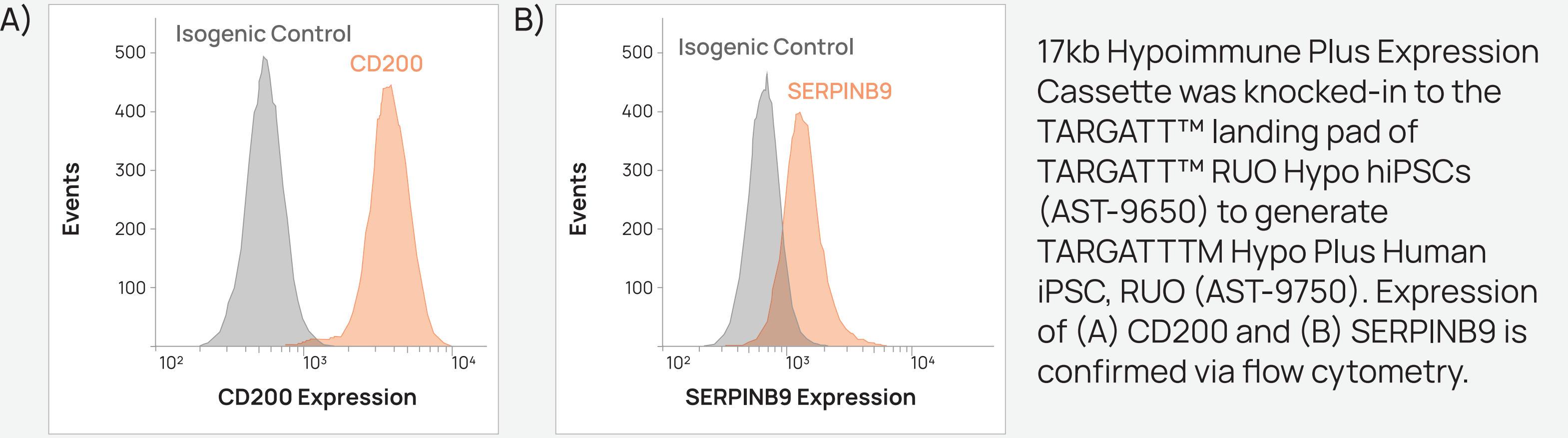


Figure 5: Confirmed Expression of Pluripotency Markers

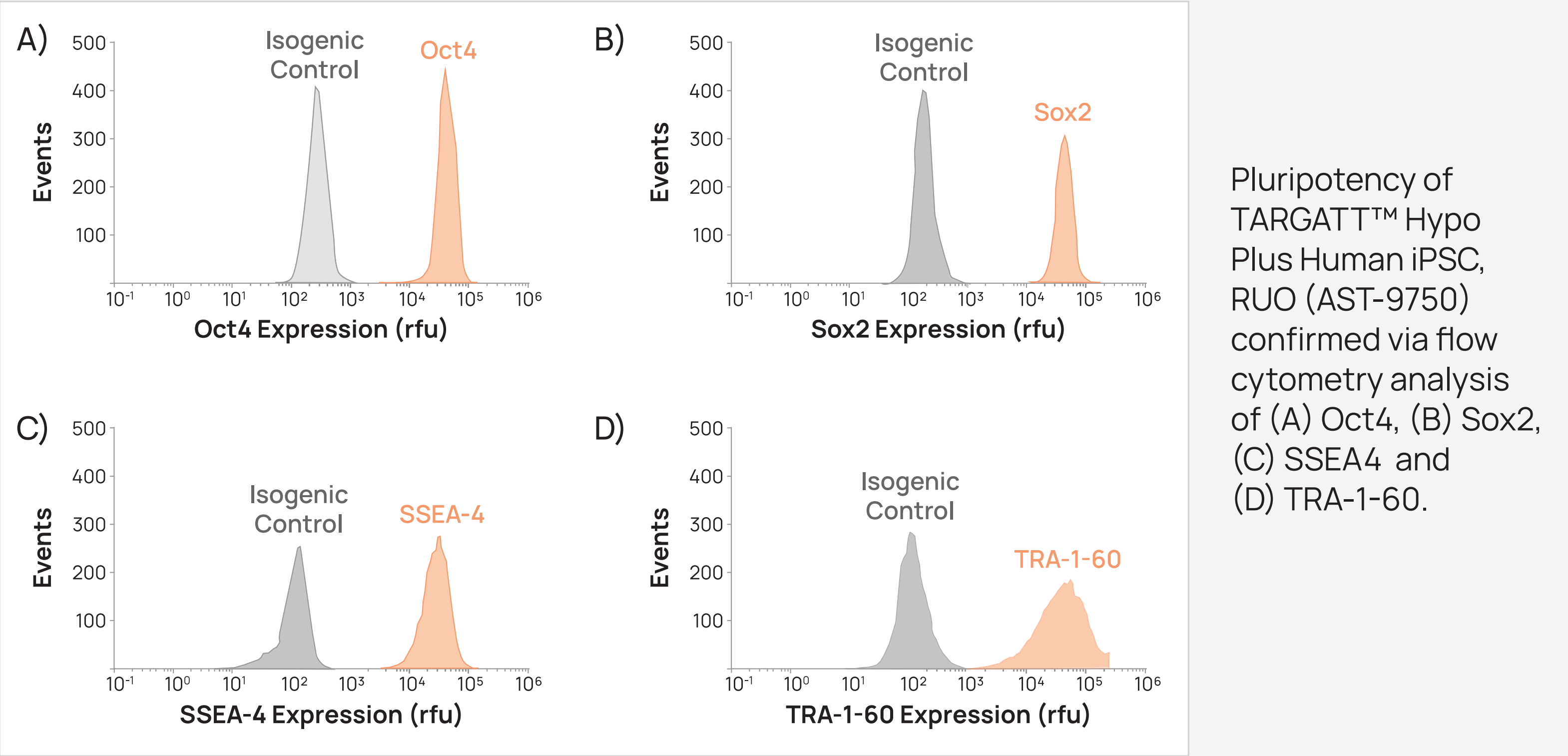
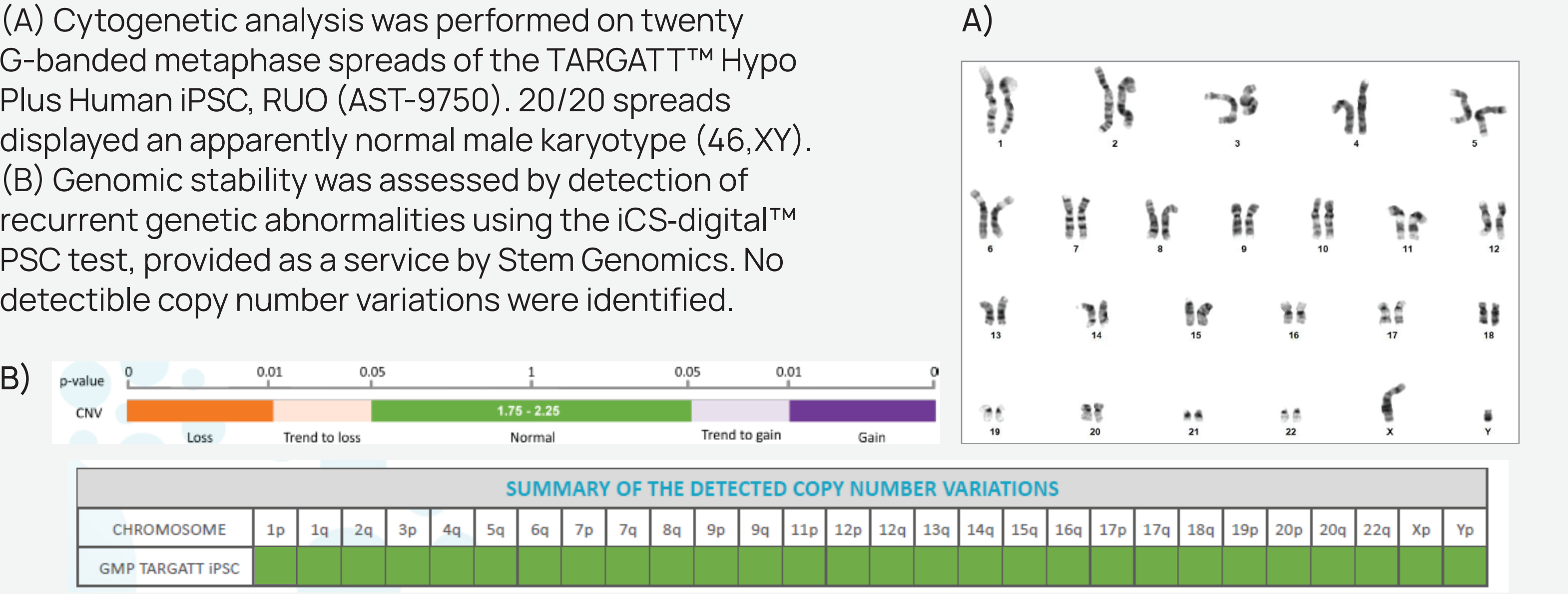


Figure 6: Karyotyping and Copy Number Variation is Normal



Citations:

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