

# Supplementary information

## Lateral plate mesoderm cell-based organoid system for NK cell regeneration from human pluripotent stem cells

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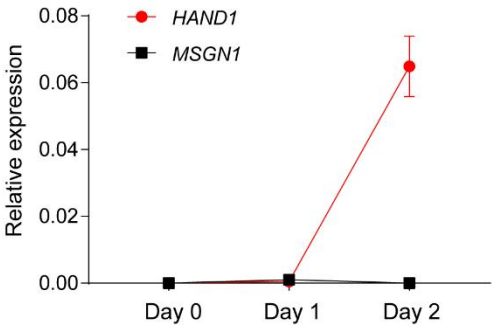
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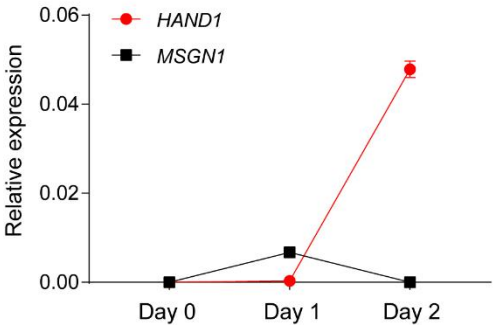
34 **Supplementary Figures and Figure legends**

**Supplementary Fig.S1**

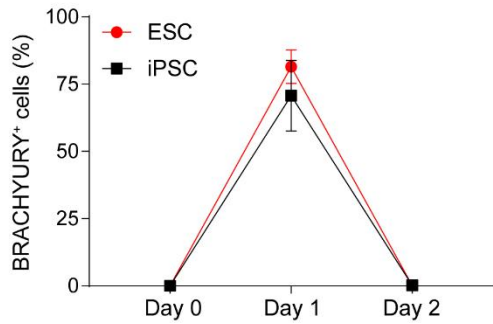
**a**



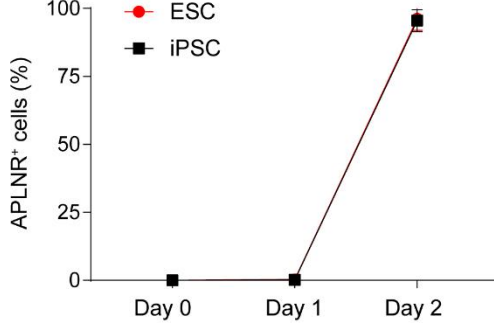
**b**



**c**



**d**



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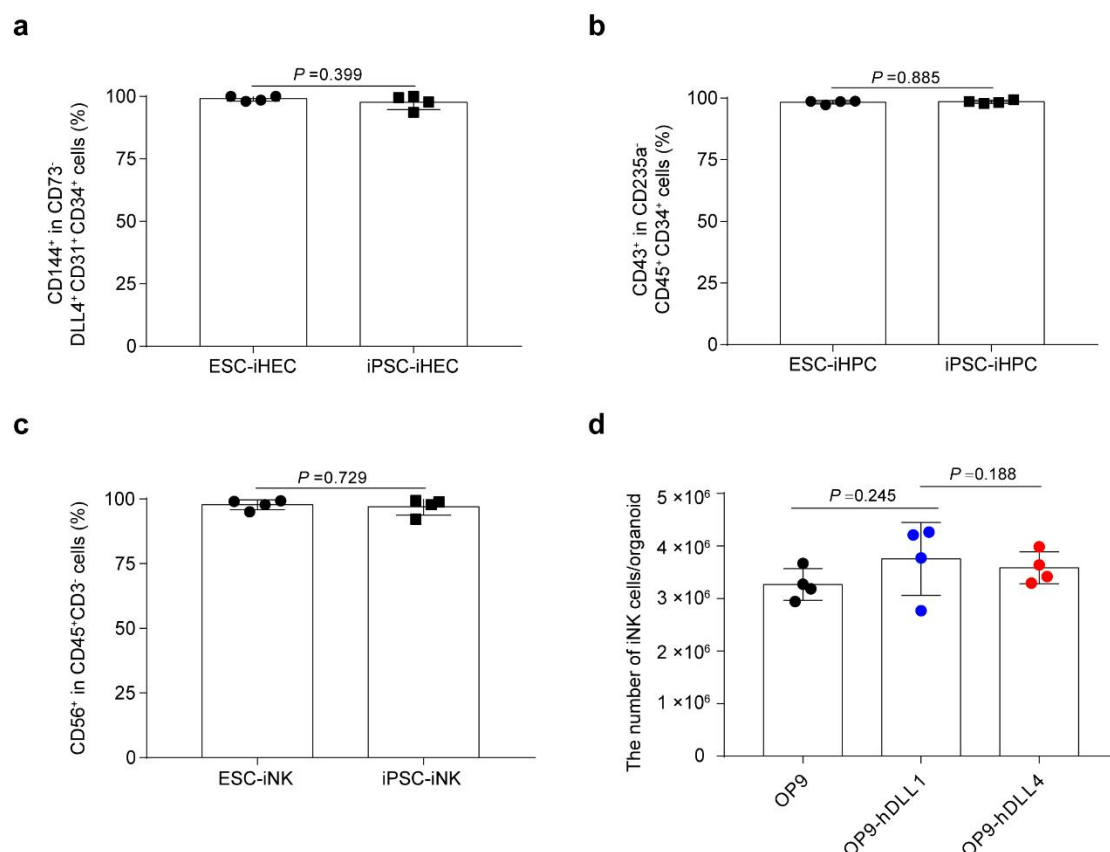
36 **Supplementary Fig. S1 Analysis of primitive streak and lateral plate**

37 **mesoderm-specific markers of hPSC-derived cells**

38 **a** Real-time quantitative PCR analysis of *HAND1* and *MSGN1* gene expression levels  
 39 of ESC-derived cells on Day 0, Day 1, and Day 2. **b** Real-time quantitative PCR  
 40 analysis of *HAND1* and *MSGN1* gene expression levels of iPSC-derived cells on Day  
 41 0, Day 1, and Day 2. **c** The proportion of BRACHYURY<sup>+</sup> cells in hPSC-derived cells  
 42 on Day 0, Day 1, and Day 2. Data were collected from three independent experiments.  
 43 *n* = 3. **d** The proportion of APLNR<sup>+</sup> cells in hPSC-derived cells on Day 0, Day 1, and  
 44 Day 2. Data were collected from three independent experiments. *n* = 3. ESC: human  
 45 ESC line hPSC-2; iPSC: human iPSC line hPSC-6.

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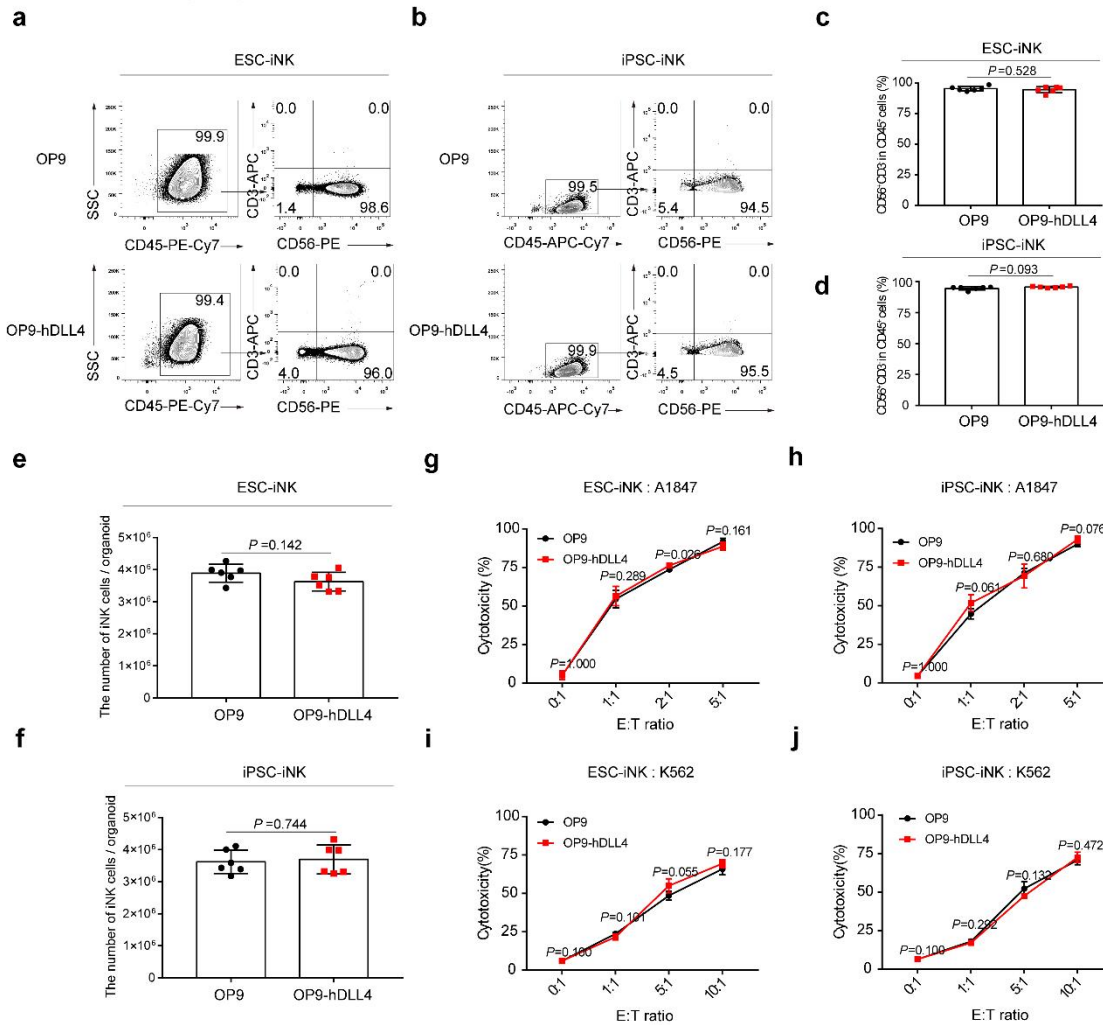
## Supplementary Fig.S2



### Supplementary Fig. S2 Analysis of the efficiency of iHEC, iHPC, and iNK cell regeneration from hPSCs

**a** The proportion of CD144<sup>+</sup> cells in CD73<sup>-</sup>DLL4<sup>+</sup>CD31<sup>+</sup>CD34<sup>+</sup> iHECs on Day 9. Data were collected from four independent experiments and analyzed by two-tailed independent *t*-test.  $n = 4$ . **b** The proportion of CD43<sup>+</sup> cells in CD235a<sup>-</sup>CD45<sup>+</sup>CD34<sup>+</sup> iHPCs on Day 16. Data were collected from four independent experiments and analyzed by Mann-Whitney U test.  $n = 4$ . **c** The proportion of CD56<sup>+</sup> cells in CD45<sup>+</sup>CD3<sup>-</sup> cells on Day 27. Data were collected from four independent experiments and analyzed by two-tailed independent *t*-test.  $n = 4$ . **d**  $5 \times 10^5$  OP9, OP9-hDLL1, and OP9-hDLL4 feeder cells were combined with  $2 \times 10^4$  mesoderm cells separately to form organoid aggregates on Day 2. Data were collected and analyzed on Day 27 ( $n = 4$  each group). Statistics: two-tailed independent *t*-test.

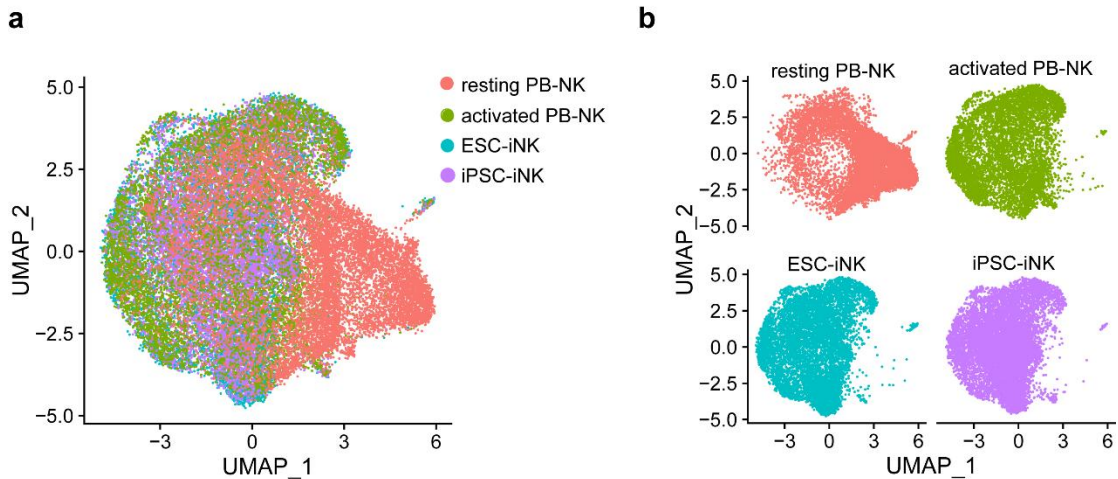
# Supplementary Fig.S3



**Supplementary Fig. S3** Comparison of the induction efficiency, maturation, and cytotoxicities of iNK cells from ESCs and iPSCs using OP9 and OP9-hDLL4 feeder cells.

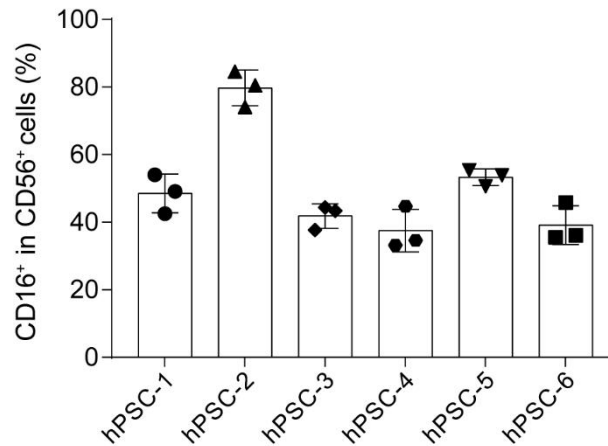
**a-b** Immuno-phenotypes (CD45<sup>+</sup>CD3<sup>+</sup>CD56<sup>+</sup>) of iNK cells from ESC (**a**) and iPSCs (**b**) on Day 27. **c-d** Statistics of the maturation of iNK cells from ESC (**c**) and iPSCs (**d**) on Day 27 ( $n = 6$  each group). **e-f** Statistics of the number of CD45<sup>+</sup>CD3<sup>+</sup>CD56<sup>+</sup> iNK cells on Day 27 ( $n = 6$  each group). **g-h** Cytotoxicity analysis of ESC-iNK cells (**g**) and iPSC-iNK cells (**h**) against A1847 cell line ( $n = 4$  each group). **i-j** Cytotoxicity analysis of ESC-iNK cells (**i**) and iPSC-iNK cells (**j**) against k562 cell line ( $n = 4$  each group). Statistics: two-tailed independent  $t$ -test (**c-j**) and Mann-Whitney U test (**g**).

## Supplementary Fig.S4



**Supplementary Fig. S4** Integration of scRNA-seq data generated from ESC-iNK, iPSC-iNK, resting PB-NK, and activated PB-NK cells. **a** Projection of ESC-iNK, iPSC-iNK, resting PB-NK, and activated PB-NK cells. **b** UMAP visualization of ESC-iNK, iPSC-iNK, resting PB-NK, and activated PB-NK cells respectively.

## Supplementary Fig.S5



**Supplementary Fig. S5** CD16 molecule expression pattern of iNK cells.

CD16 expression levels of iNK cells and NK cells from human embryonic cell line hPSC-1, hPSC-2, hPSC-3, hPSC-4, hPSC-5, and human iPSC line hPSC-6. CD16<sup>+</sup> cells were gated on CD45<sup>+</sup>CD3<sup>-</sup>CD56<sup>+</sup> iNK cells. Data were collected from three independent experiments. All samples were collected on Day 27.

88 **Supplementary Table S1**

Primer sequences for real-time quantitative PCR of selected genes	
Primer name	Primer sequence (5'→3')
<i>β-ACTIN</i> -F	CATGTACGTTGCTATCCAGGC
<i>β-ACTIN</i> -R	CTCCTTAATGTCACGCACGAT
<i>TBXT</i> -F	TATGAGCCTCGAATCCACATAGT
<i>TBXT</i> -R	CCTCGTTCTGATAAGCAGTCAC
<i>APLNR</i> -F	CTCTGGACCGTGTTTCGGAG
<i>APLNR</i> -R	GGTACGTGTAGGTAGCCCACA
<i>HAND1</i> -F	GAGAGCATTAACAGCGCATTTCG
<i>HAND1</i> -R	CGCAGAGTCTTGATCTTGGAGAG
<i>MSGNI</i> -F	AACCTGCGCGAGACTTTCC
<i>MSGNI</i> -R	GTCTGTGAGTTCCCCGATGTA

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