Supplementary Material

Materials and Methods

Primer sequences and binding sites

CD44s: 5'- AGCAGCGGCTCCTCCAGTGA, binds within standard exon 5. 5'- CCCACTGGGGTGGAATGTGTCT, binds within standard exon 6.

CD44v3: 5'- GCACTTCAGGAGGTTACATC, binds within standard exon 5. 5'- CTGAGGTGTCTGTCTCTTTC, binds within variant exon v3.

CD44v6: 5'- AGGAACAGTGGTTTGGCAAC, binds within variant exon v6. 5'- CGAATGGGAGTCTTCTTTGG, binds within variant exon v6.

CD44v10: 5'- GGAATGATGTCACAGGTGGA, binds within variant exon v10. 5'- AGGTCACTGGGATGAAGGTC, binds within variant exon v10.

Run-on assay

mRNA stability assay was performed as previously described[1].

mRNA stability assay

mRNA stability assay was performed as previously described[2] with a minor modification: expression of 18S was used as the control.

References

1. Zhuo DX, Niu XH, Chen YC, et al. Vitamin D3 up-regulated protein 1(VDUP1) is regulated by FOXO3A and miR-17-5p at the transcriptional and post-transcriptional levels, respectively, in senescent fibroblasts. J Biol Chem. 2010; 285: 31491–501.
2. Patrone G, Puppo F, Cusano R, et al. Nuclear run-on assay using biotin labeling, magnetic bead capture and analysis by fluorescence-based RT-PCR. BioTechniques. 2000; 29: 1012–4, 1016–7.

Appendix

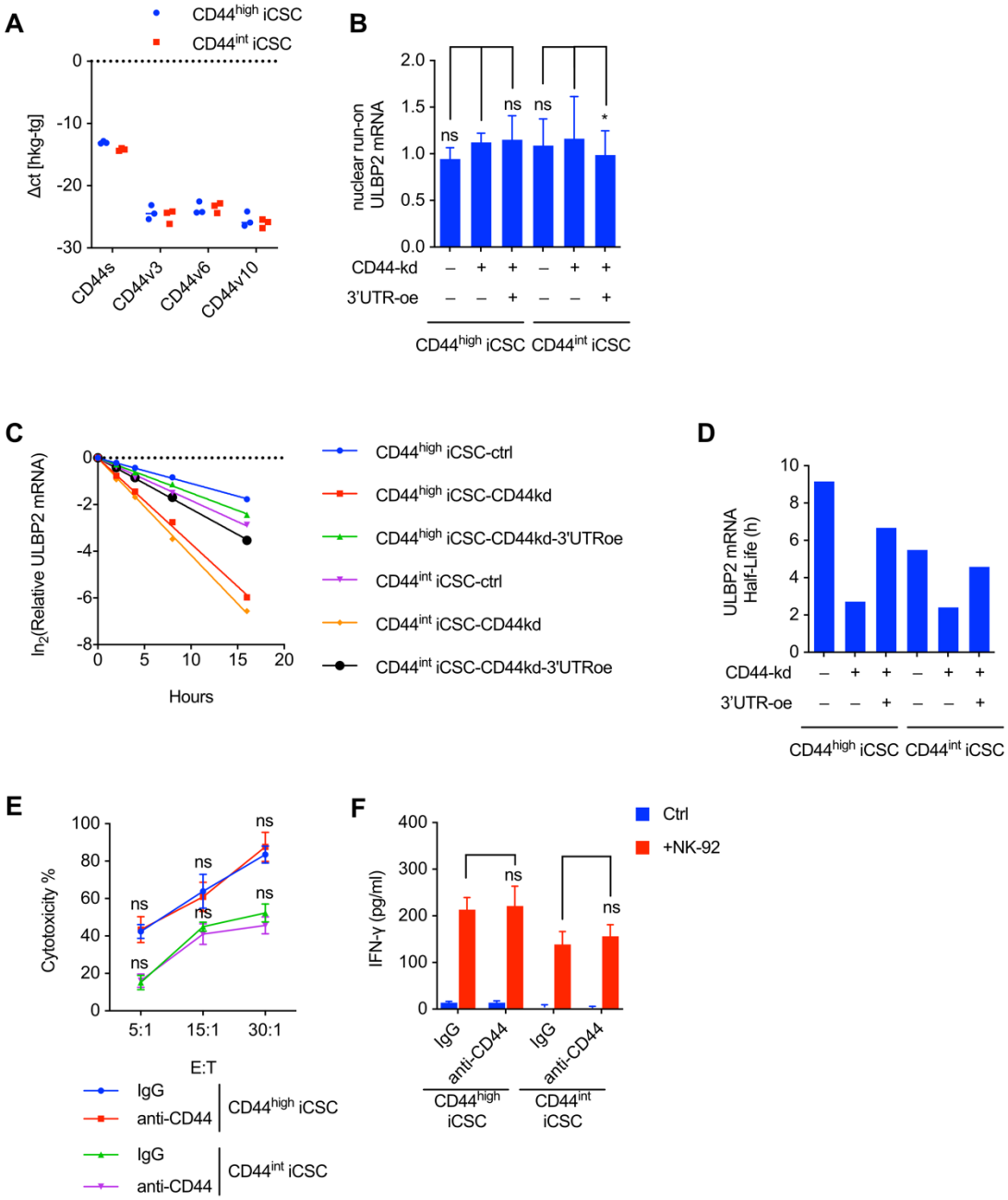

Fig. S1

Figure Legends

(A) The transcript level of CD44s and CD44v in CD44^{high}iCSC/CD44^{int}iCSC were analyzed by qRT-PCR. Each dot represents one replicate. Δct values (ct housekeeping gene [hkg] minus ct target gene [tg]) of three biological replicates were shown.

(B) CD44^{high}iCSC/CD44^{int}iCSC stably expressing dCas9-KRAB and sgRNA against CD44 (CD44-knockdown, CD44-kd) were transfected with blank expression vector (Ctrl) or CD44 3'UTR expression

vector (CD44 3'UTR -overexpression, 3'UTR-oe). Nuclear run-on assay of ULBP2 promoter was performed; data were presented as means mean ± SD (n=3) (ns: no significance, *: $p < 0.05$).

(C) mRNA stability assay was performed in identical cells (Fig. S1B).

(D) Half-life of ULBP2 mRNA was calculated based on mRNA stability assay.

(E) $CD44^{high}$iCSC and $CD44^{int}$iCSC were subjected to the NK cell cytotoxicity assay with NK-92 cells at different E:T ratios for 4 hours. Assays were performed either in the presence of anti-CD44 mAb or control IgG. Data were shown in means ± SD from three independent experiments which were performed in triplicate (ns: no significance).

(F) The IFN-γ release in the supernatants of cytotoxic assay (Fig. 2C, E:T ratio = 15:1) was determined by ELISA. Data were presented as mean ± SD (n=3) (ns: no significance).