Background
Extensive data on gene expression, metabolic state and glycosylation of cancer cells suggest that cancer represents a reversion of adult cells to an embryonic state and that induced pluripotent stem cells (iPSC) phenocopy this state. In contrast to cancer cells, iPSC have never undergone immunediting and therefore present hundreds of oncofetal antigens in their native conformations. Previous studies have demonstrated the efficacy of syngeneic iPSC vaccination in a variety of cancer models. In this study, we administered vaccines comprising live or dead iPSC either in PBS or in the cryopreservative CryoStor CS10 together with the Toll-like receptor (TLR) 9 agonist CpG1826 (CpG) as an adjuvant and compared their immunogenicity and preclinical efficacy in a prophylactic mouse model of breast cancer.

Methods
FVB mice (n=10) received a course of 4 weekly s.c. injections with (i) live, freshly harvested iPSC in PBS; (ii) live, cryopreserved iPSC in CryoStor; (iii) dead iPSC in PBS; and (iv) dead iPSC in CryoStor (each admixed with 1nmol CpG) or (v) CpG alone as control. All iPSC were irradiated with 60 Gy and the dose per injection was 10^7 cells. Cell death was induced by three freeze-thaw cycles between -195°C and 37°C. One week after the 4th treatment, serum was obtained for IgG binding studies and 5x10^5 DB7 syngeneic breast cancer cells were injected into the mice s.c. Tumor growth was monitored for 24 days post tumor cell injection.

Results
In this prophylactic syngeneic breast cancer model each of the four different iPSC+CpG vaccines significantly reduced tumor growth compared to the treatment with the adjuvant CpG alone (all p<0.03, 2-way ANOVA with Tukey’s multiple comparisons test) (figure 1). No statistically significant difference was observed between live and dead iPSC or between iPSC applied in PBS or CryoStor (all p>0.74). Similar, there was no significant difference in the induction of DB7-specific IgG antibodies between the four iPSC vaccine treatments. IgG binding to DB7 cells inverse correlated with tumor size on day 24 (r=-0.31, p=0.035).

Conclusions
Antigen cross-presentation is independent of whether the iPSC are alive or dead. Cryopreservation of iPSC and viability have no negative impact on the immunogenicity and efficacy of our iPSC vaccine. This simplifies the manufacture, storage and shipment of the vaccine and eases clinical application.

Ethics Approval
The study was approved by Valley Bio Services’ Institutional Animal Care and Use Committee; approval number VBS1002.