# ImmTAC redirect T cells against patient-derived tumour organoids and three-dimensional melanospheres; effects augmented by type I interferons

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Background

- Immune mobilizing monoclonal T cell receptors (TCRs) Against Cancer (ImmTAC®) are soluble bispecific biologics that redirect T cells to kill tumor targets by binding to both CD3 on T cell surface and specific peptide-HLA (pHLA) on the tumor[1] (Figure 1). Tebentafusp, which targets gp100 (gp100 × CD3), is the first ImmTAC to demonstrate overall survival benefit and is approved for the treatment of metastatic uveal melanoma in HLA-A*02:01+ patients[2].

- Here, patient-derived tumour organoids (TO)[3] and 3-dimensional multicellular melanospheres were evaluated as tumour models to study ImmTAC activity.
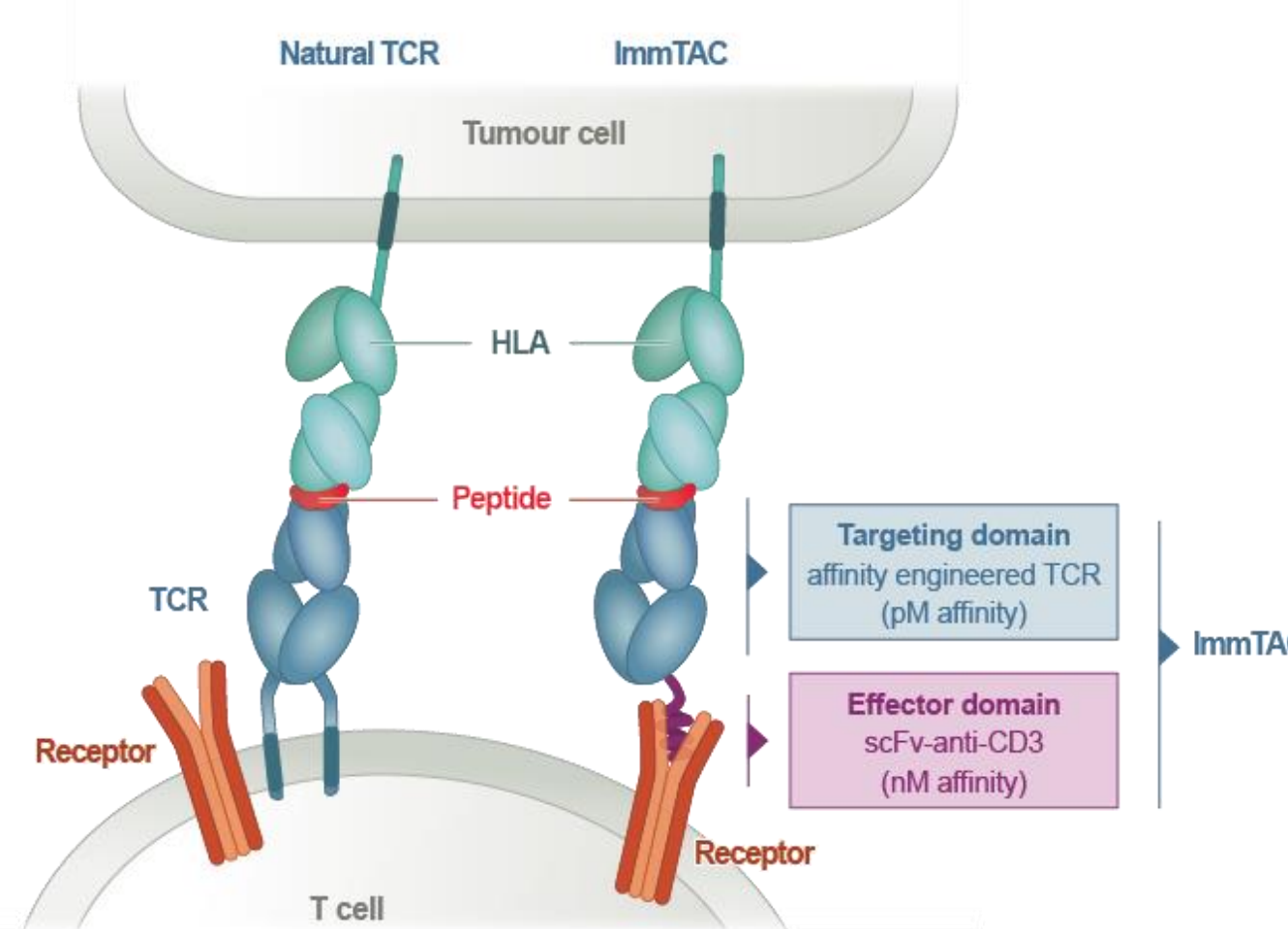

Figure 1. ImmTAC molecules mimic the immune synapse formed by a natural T cell-tumour cell interaction.

Methods

- 3D melanospheres were generated by low adherence culture of melanoma cell lines (MEL202, 92.1, MP-41, A375, IGR-1, Mewo). 3D melanospheres were generated after at least 8 days in culture.

- Melanin synthesis genes (PMEL, MLANA, TYR, and MITF) were quantified by qPCR, whilst melanin was visualised by H&E staining of 3D cultures.

- T cell redirection against 3D melanospheres was assessed by IFNγ Elispots using the gp100 targeting research tool ImmTAC.

- Patient derived TO were generated by Tempus[3] from liver, lung, and head & neck HLA-A*02:01+ cancer patients. TO were screened for Ag expression and relevant ImmTACs were used to re-direct T cell killing of Ag+ TO, measured by 3D high content imaging.
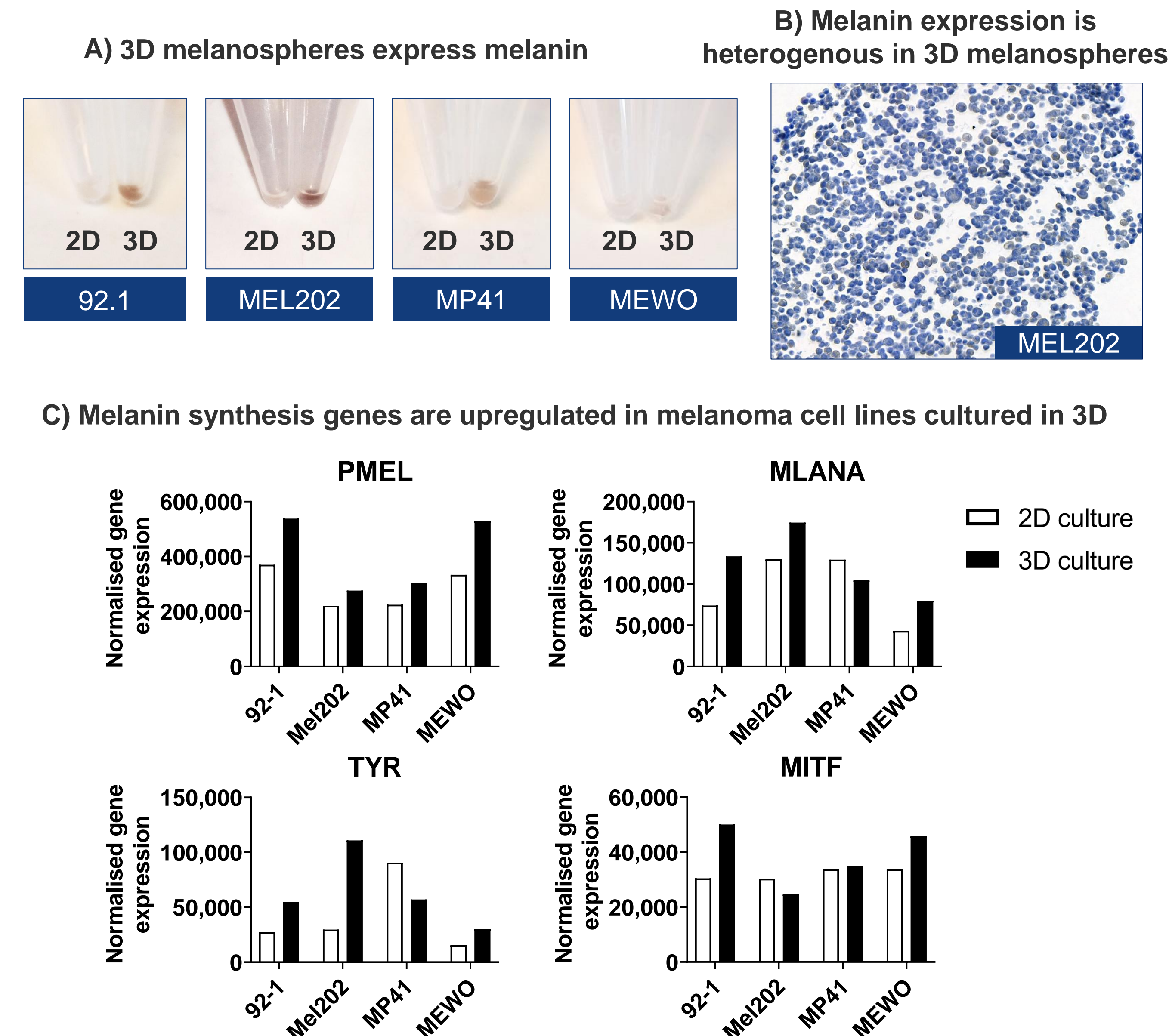
Results

### Generation of 3-dimensional melanosphere structures from cutaneous and uveal melanoma cell lines
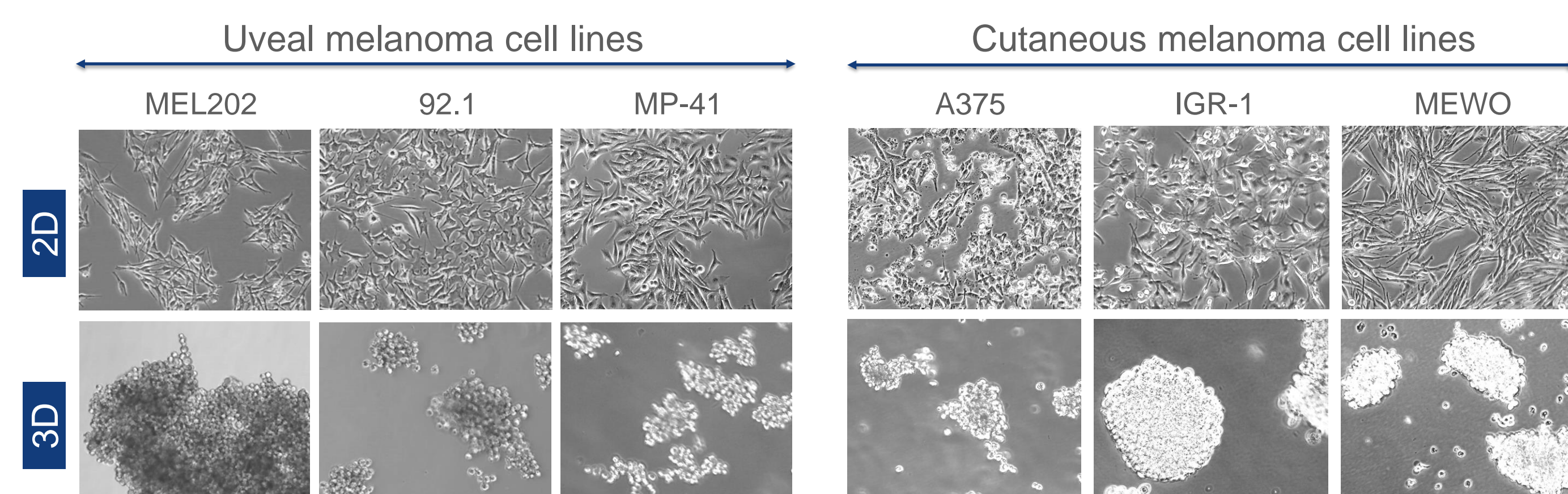
Figure 2. Uveal and melanoma cell lines were cultured as adherent monolayers (top row) or in low adherence conditions (bottom row) for 8 days. Representative phase contrast images are shown (x20 magnification).

3D Melanospheres upregulate melanin synthesis machinery

A) 3D melanospheres express melanin

B) Melanin expression is heterogenous in 3D melanospheres

C) Melanin synthesis genes are upregulated in melanoma cell lines cultured in 3D

Figure 3. Melanoma cell lines upregulate melanin synthesis in 3D: A) Pellets from indicated cell lines showing visible melanin production. B) H&E staining of MEL202 cultured in 3D showing melanin expression heterogeneity. C) Melanin synthesis genes were quantified by qPCR in 2D (clear bars) or 3D (black bars) cultures.

Gp100 ImmTAC redirects T cells against gp100+ melanospheres, effects augmented by type I IFN
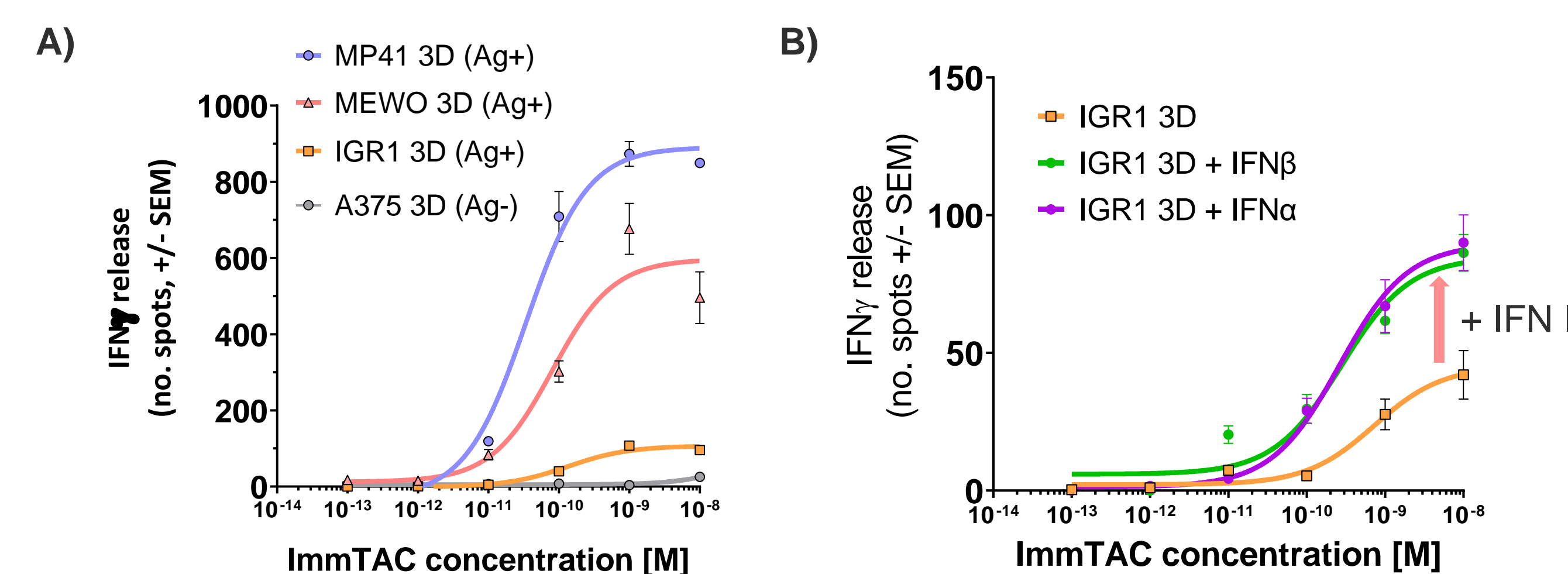

Figure 4. 3D melanospheres were generated from gp100 positive and negative melanoma lines. Their ability to redirect gp100 ImmTAC to induce T cell activation was measured by IFNγ Elispots. A) Responses were observed in all gp100+ but not gp100- cell lines tested. B) Type I interferon treatment of 3D cell lines for 72 hours augmented their ability to redirect ImmTAC-mediated T cell activation.
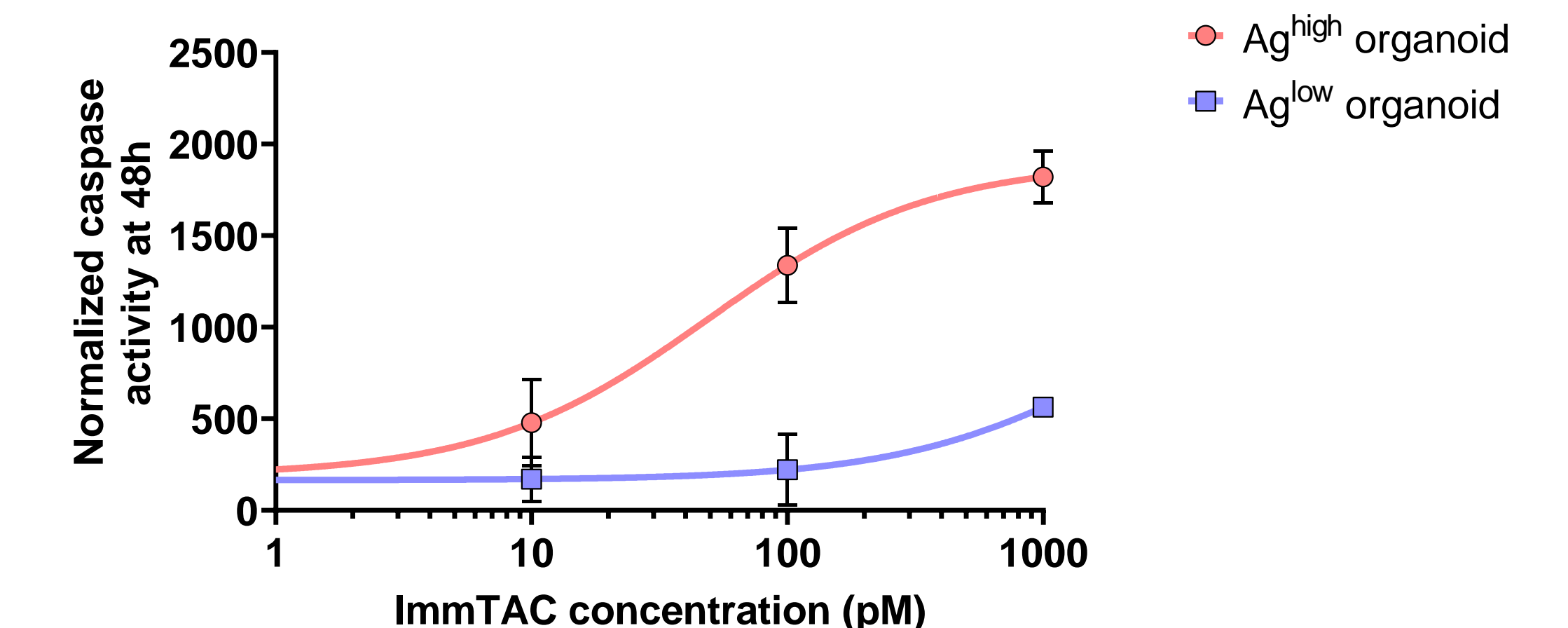
ImmTAC redirects T cells to kill antigen-positive patient-derived tumor organoids
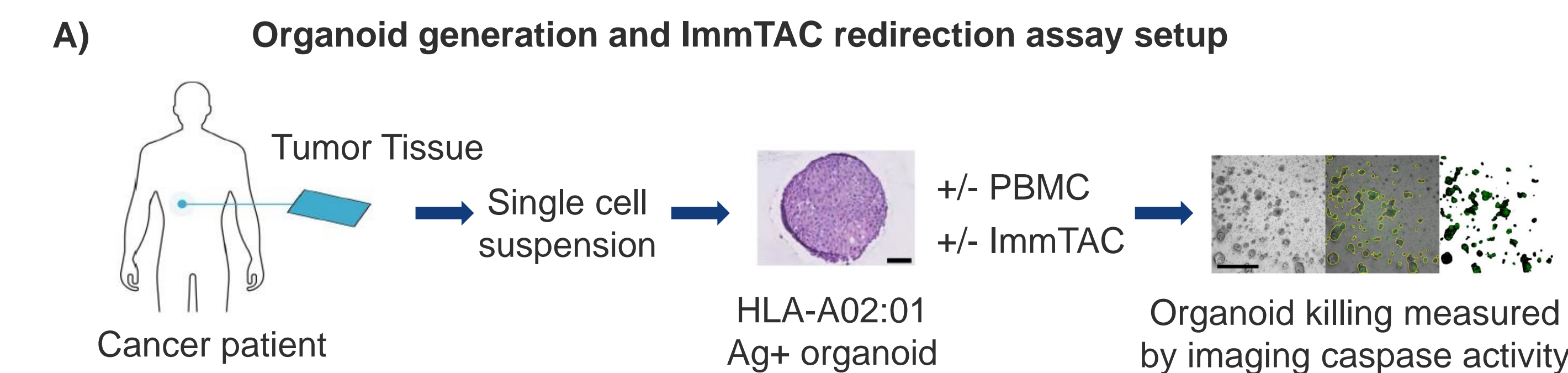
A) Organoid generation and ImmTAC redirection assay setup

B) Time-course of ImmTAC-specific killing of an antigen-high organoid
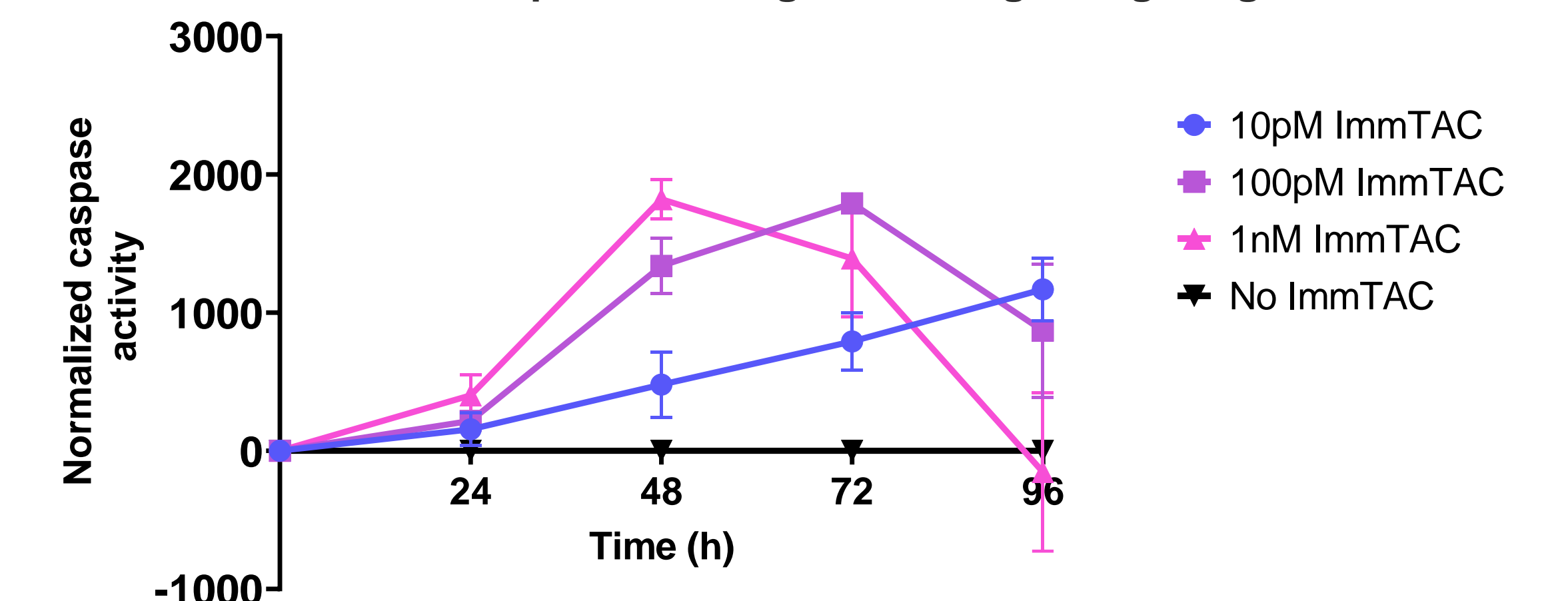
C) Dose-dependent killing was observed in organoids with high antigen expression

Figure 5. ImmTAC-directed T cell killing of patient-derived tumour organoids. A) Schematics of organoid generation and ImmTAC killing assays. B) Time-course of ImmTAC-specific killing of an antigen-high organoid. C) Dose-dependent killing was observed in organoids with high antigen expression

Conclusions

- 3D melanospheres express melanin and can mimic tumour heterogeneity observed in vivo.

- ImmTAC were capable of redirecting T cells against 3D melanospheres and patient-derived tumour organoids in a dose dependent and antigen dependent manner.

- 3D melanospheres and organoids are useful models to study tumour biology, heterogeneity and ImmTAC mechanism of action.

References

1. Lowe et al. (2019) Cancer Treat Rev. Vol.77, p35-43

2. Nathan P et al. N Engl J Med. 2021. 1196-1206.3.

3. Larsen B et al. Cell Rep. 2021; 36(4).

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