Co-expression of the metabolic enzyme GOT2 with a GPC3-targeted CAR-T overcomes the challenges of the solid tumor microenvironment, substantially improving therapeutic efficacy in solid tumor xenografts

#P227

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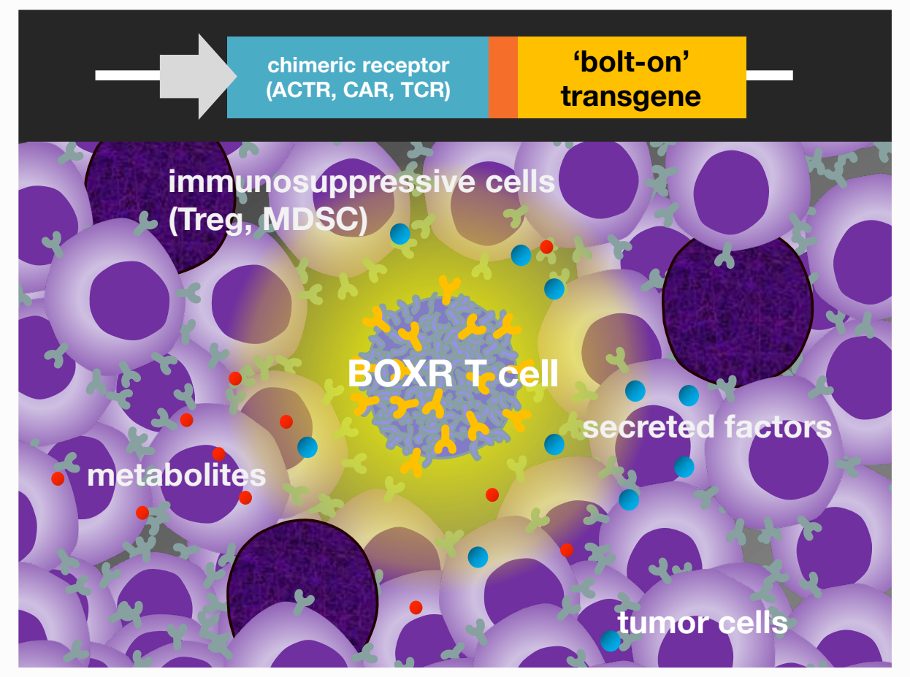
UNUM THERAPEUTICS

Introduction

The metabolic demands of cancer cells in the solid tumor microenvironment (TME) create an unfavorable T cell environment through depletion of critical nutrients and amino acids and accumulation of waste products. This drives T cell dysfunction and inhibits the effectiveness of immunotherapies. To overcome these and other TME challenges, we developed the BOXR (bolt-on chimeric receptor) platform in which engineered T cells co-express both a chimeric-targeting receptor and a "bolt-on" transgene. In a screen of 100+ genes for enhanced T cell function when co-expressed with an anti-glypican-3 (GPC3) CAR, we identified the first candidate of our BOXR platform, BOXR1030, which co-expresses the transgene glutamic-oxaloacetic transaminase 2 (GOT2), a critical enzyme involved in mitochondrial metabolism. Here, we present preclinical characterization of the mechanism of action of BOXR1030.

The BOXR platform

BOXR T cells overcome immune suppression in the TME



BOXR components

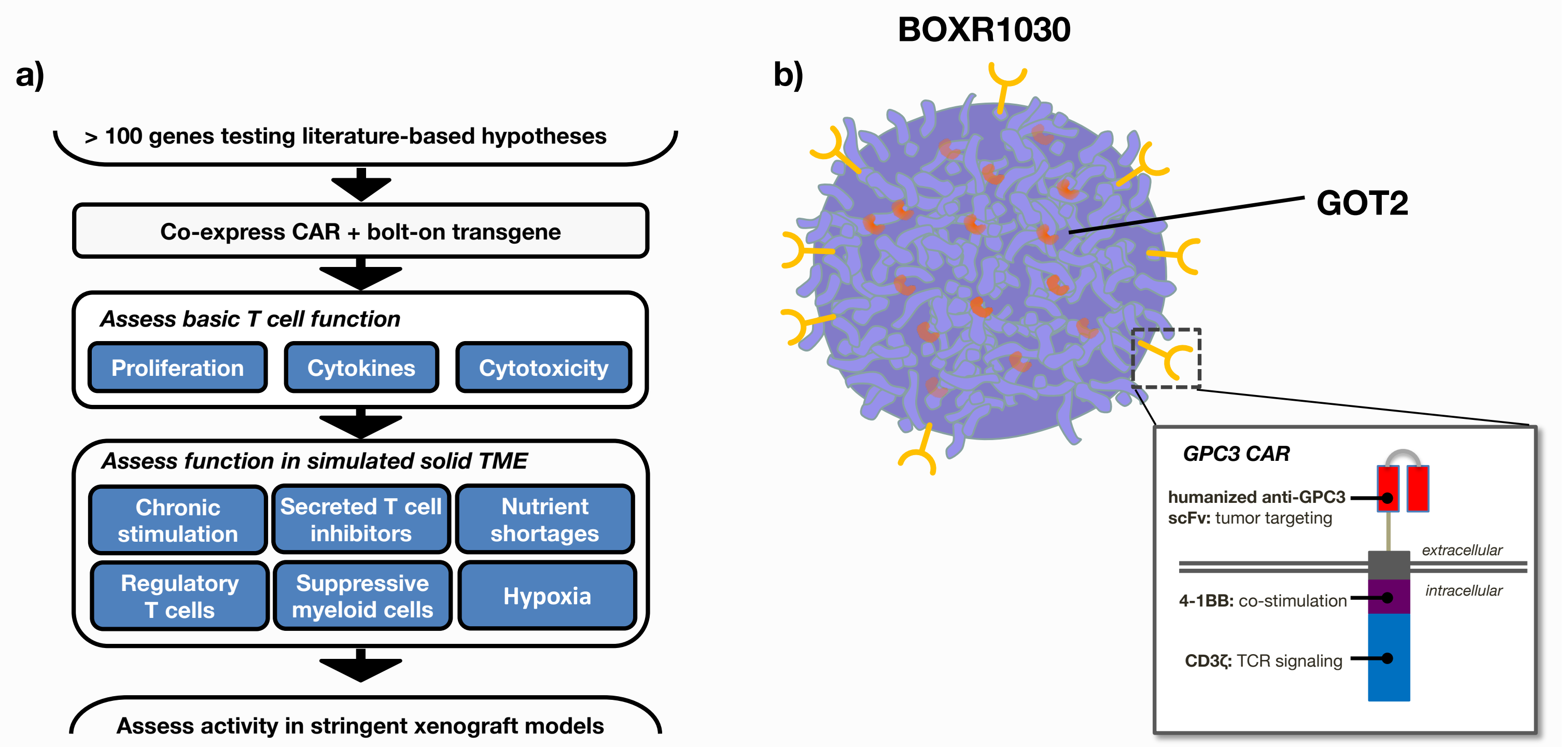
- **chimeric receptor:** Tumor targeting modality (CAR, ACTR, TCR, etc.) drives cancer cell targeting and attack
- **bolt-on:** novel transgene re-programs T cell biology to improve functionality in the tumor microenvironment

Targeting key mechanisms of immunosuppression

- Competition for metabolites
- Immunosuppressive cells (MDSC, Treg)
- Exhaustion due to chronic stimulation

Discovery of BOXR1030

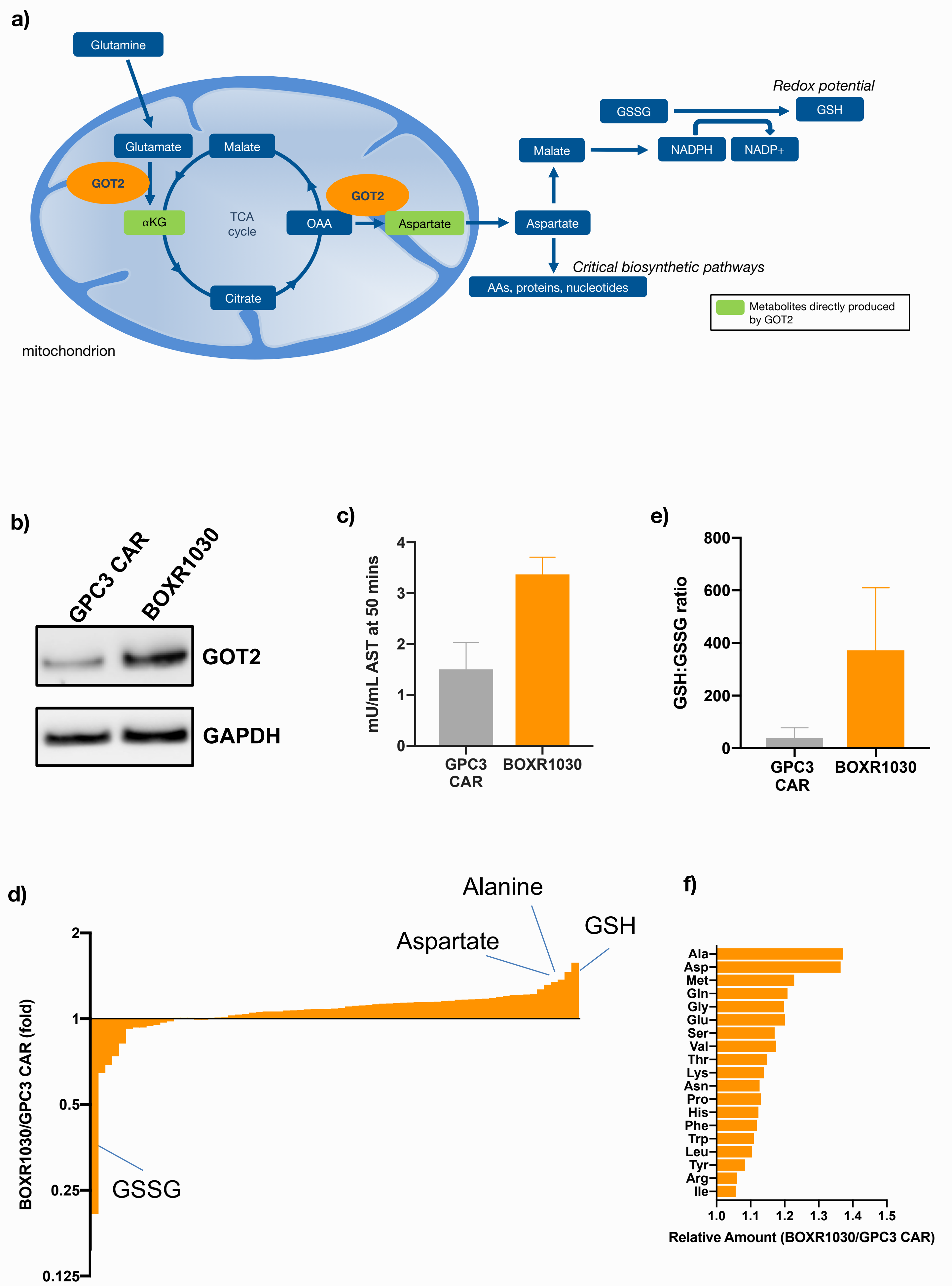
Screening of 100+ of bolt-ons led to the discovery of BOXR1030



a) BOXR candidates were generated by cloning a library of literature-derived, hypothesis-driven bolt-on genes into vectors containing a GPC3-targeted CAR that had either a 4-1BB or CD28 co-stimulation domain. BOXR constructs were screened through Unum's novel solid tumor microenvironment assays, followed by assessment of activity in stringent xenograft models. Candidates were selected on their ability to overcome multiple TME-challenges, while maintaining specificity and tolerability. BOXR1030 was selected from this screening. b) BOXR1030 is a GPC3-targeted CAR that contains a humanized scFv, a 4-1BB co-stimulation domain and co-expresses the transgene glutamic-oxaloacetic transaminase 2 (GOT2), a critical enzyme involved in cellular metabolism.

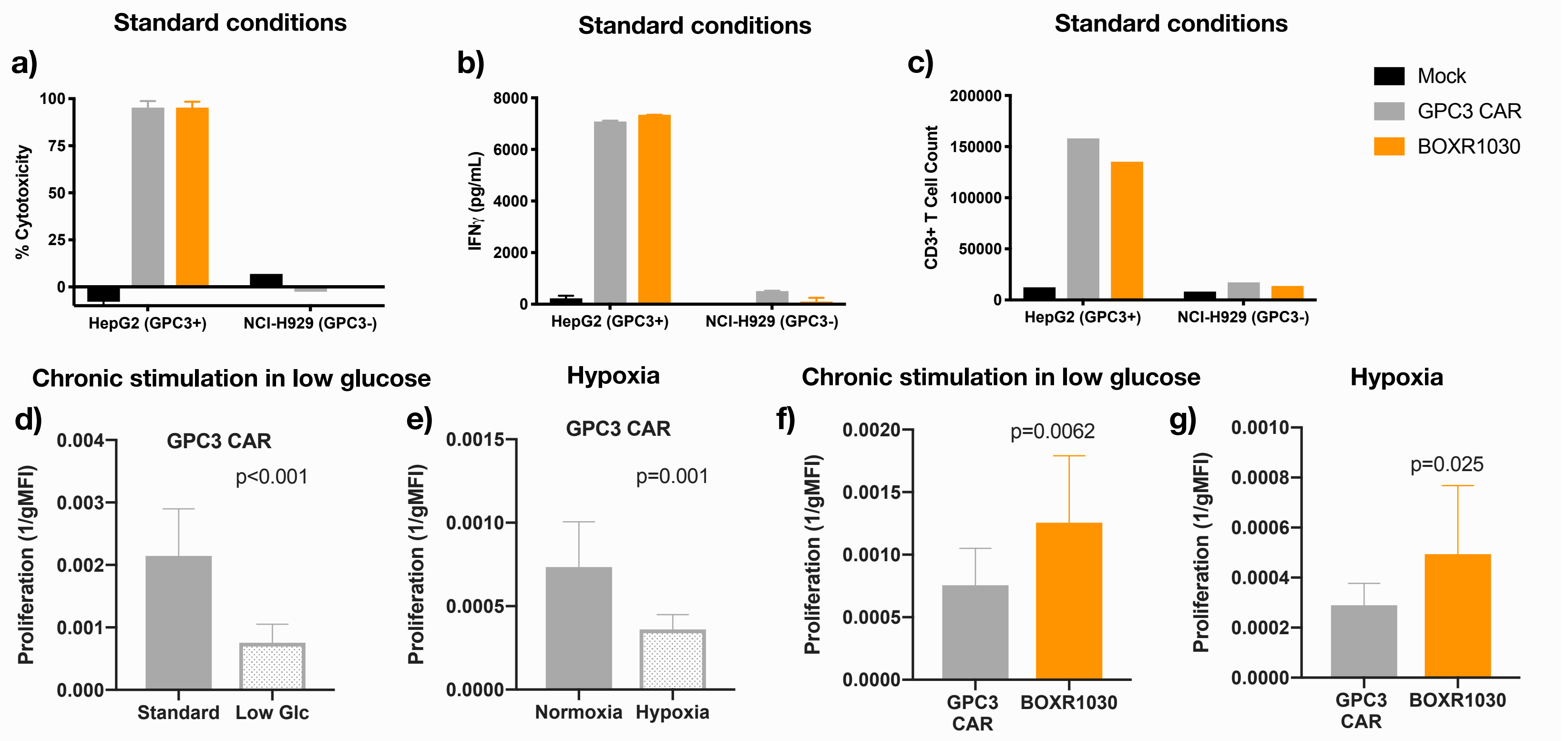
Results

GOT2 overexpression increases critical metabolites in T cells



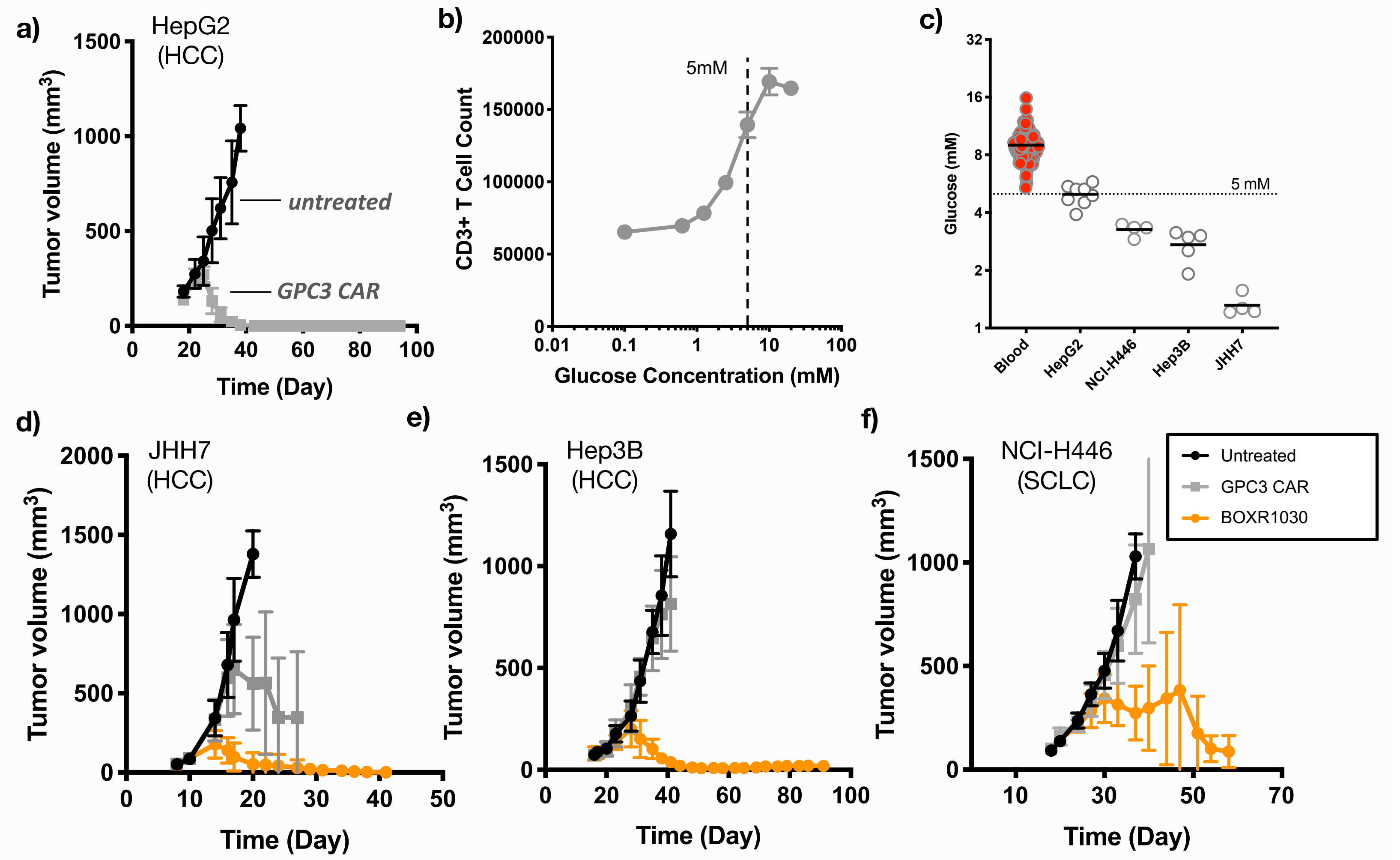
a) Depiction of biochemical steps related to GOT2 activity. b) Western blot analysis of a control GPC3 CAR and BOXR1030 T cells for GOT2 following activation with Hep3B GPC3+ target cells. GADPDH was used as a loading control. c) Aspartate aminotransferase activity of BOXR1030 compared to a control GPC3 CAR. Lysates were evaluated following activation with Hep3B GPC3+ target cells. d) A targeted panel of 116 metabolites involved in energy metabolism was evaluated by CE-MS for both BOXR1030 and a control GPC3 CAR. Means of metabolite values (pmol/10e6 cells) from 2 unique donors were pooled and plotted as a ratio of BOXR1030 relative to a GPC3 CAR. Metabolites of interest are highlighted. e) The ratio of glutathione (GSH) to glutathione disulfide (GSSG) is depicted from the metabolite analysis described. f) Amino acids detected from the metabolic profile (cysteine below the limit of detection).

BOXR1030 activity is superior to a control CAR in TME-like conditions



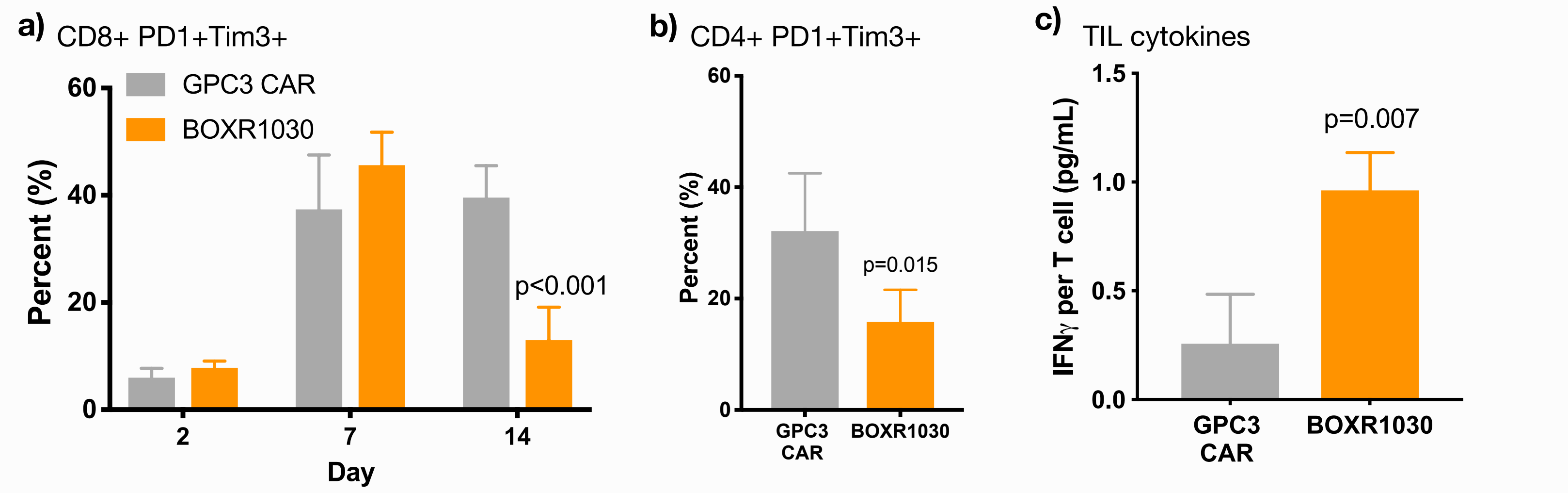
Control GPC3 CAR or BOXR1030 T cells were incubated with either GPC3+ HepG2 or GPC3-negative NCI-H929 tumor cells. 24 hours later a) cytotoxicity and b) cytokine production was measured. c) In a separate assay with similar conditions, T cell proliferation was measured on day 7. Representative data from a single donor. In a separate study, control GPC3 CAR T cells were labeled with cell trace violet, and incubated with GPC3+ Hep3B tumor cells. d) To mimic chronic stimulation under TME-like conditions, T cells were cultured in low glucose (2mM) and restimulated with targets 3 days after initial incubation or e) co-cultured in hypoxia (1.5% O_2). Similarly, control GPC3 CAR and BOXR1030 T cells were compared under identical conditions f) and g). Following 7 days, CAR+CD3+ T cells were evaluated by flow cytometry for loss of cell trace violet signal as a readout of T cell proliferation. Data are presented as means of 11 unique donors.

BOXR1030 T cells have superior antitumor activity in CAR-resistant xenograft models



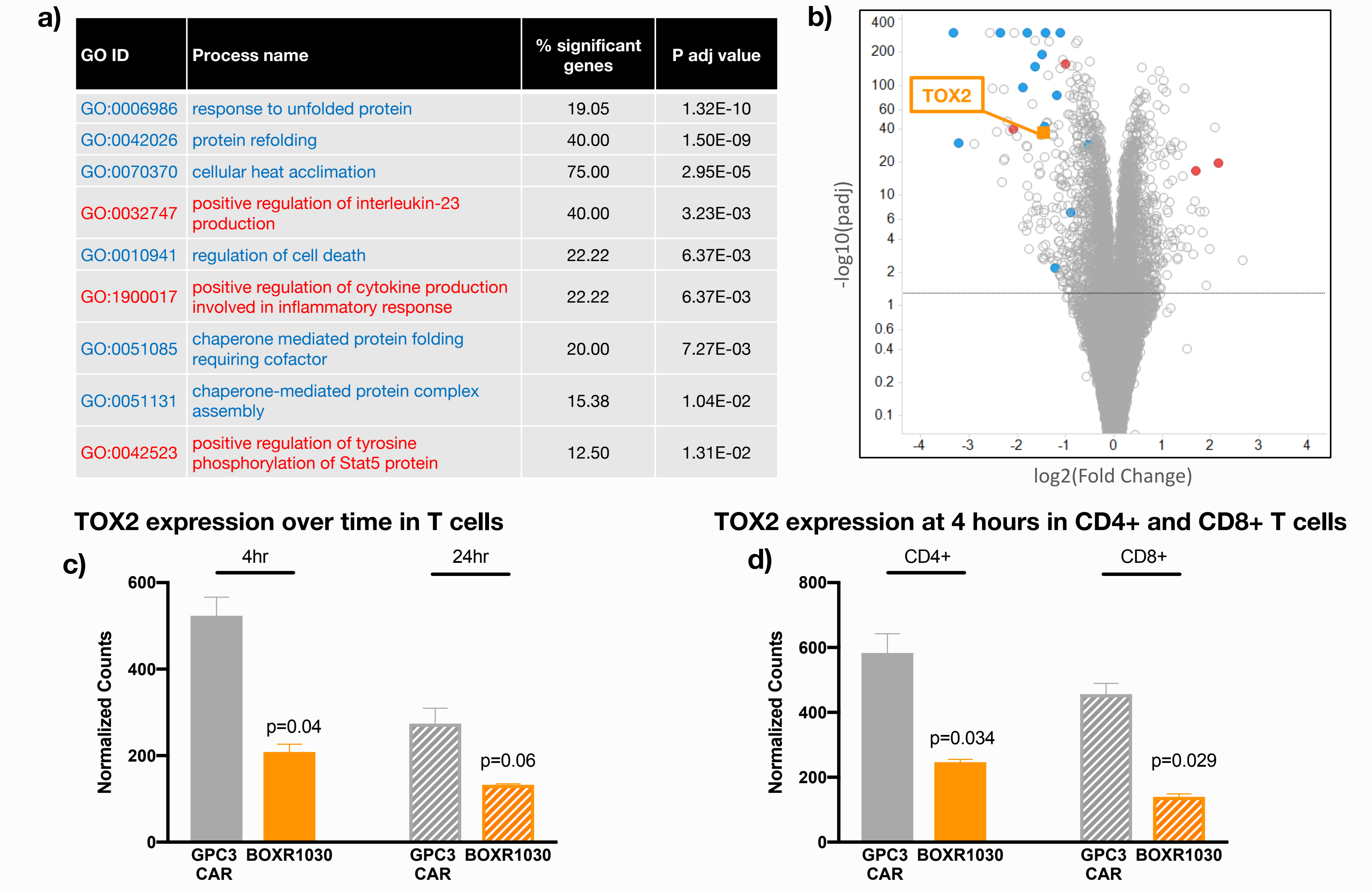
a) Mice bearing HepG2 tumors were treated with control GPC3 CAR T cells. b) Proliferation of a control GPC3 CAR was measured by flow cytometry following co-culture with GPC3+ JHH7 target cells in a dose-titration of glucose. c) Interstitial free glucose was measured from a panel of GPC3+ xenograft tumors. Mice bearing d) JHH7 e) Hep3B or f) NCI-H446 xenograft tumors were treated with either control GPC3 CAR T cells, or BOXR1030 T cells. Data shown as means (n=5/group).

BOXR1030 T cells resist TME-driven dysfunction



Mice bearing JHH7 tumors were dosed with either a control GPC3 CAR or BOXR1030 T cells. T cells were isolated and analyzed by flow cytometry or were evaluated ex vivo for cytokine production. (a) CD8+ T cells over time. Data are mean of 5 animals. (b) CD4+ T cells on day 14. Data are mean of 5 animals. (c) Cytokine production from ex vivo cultured T cells. Data are mean of 3 animals.

RNA transcriptional profile of BOXR1030 is consistent with reduced exhaustion and reduced metabolic stress



a)

GO ID	Process name	% significant genes	P adj value
GO:0006986	response to unfolded protein	19.05	1.32E-10
GO:0042026	protein refolding	40.00	1.50E-09
GO:0070370	cellular heat acclimation	75.00	2.95E-05
GO:0032747	positive regulation of interleukin-23 production	40.00	3.23E-03
GO:0010941	regulation of cell death	22.22	6.37E-03
GO:1900017	positive regulation of cytokine production involved in inflammatory response	22.22	6.37E-03
GO:0051085	chaperone mediated protein folding requiring cofactor	20.00	7.27E-03
GO:0051131	chaperone-mediated protein complex assembly	15.38	1.04E-02
GO:0042523	positive regulation of tyrosine phosphorylation of Stat5 protein	12.50	1.31E-02

Control GPC3 CAR and BOXR1030 T cells from 2 unique donors were co-cultured with immobilized GPC3 protein for 4 and 24 hours, followed by RNA extraction. RNA was then analyzed by RNA-Seq. In a parallel experiment, CD4 and CD8 T cells from a single donor were isolated for RNA analysis. a) At 4hrs, a number of GO processes were differentially expressed with high significance. Red=immune response genes; blue=cell stress genes b) At 4hours, many genes were differentially expressed and genes associated with significantly different GO processes are indicated by color. Shown is data from an individual donor. c) Notably, a key driver of T cell exhaustion, TOX2, was substantially lower in BOXR1030 from both donors at 4 and 24 hours. d) 4 hours after stimulation, TOX2 gene expression was substantially lower in BOXR1030 T cells in both CD4+ and CD8+ T cell subsets.

Conclusions

Unum's BOXR T cell platform is a novel approach to discover transgenes that overcome the challenges faced by T cells in solid tumors, and led to the discovery of BOXR1030. BOXR1030 is a GPC3-targeted CAR that co-expresses GOT2. The addition of GOT2 led to improved metabolic and transcriptional profiles associated with superior activity in the face of diverse TME challenges in vitro and in vivo. These results demonstrate that engineering of T cell immunometabolism is an effective and potent strategy to overcome the challenges of the solid tumor microenvironment and prevent T cell dysfunction in the TME. IND-enabling studies with BOXR1030 are underway with the expectation that BOXR1030 will be evaluated clinically in the treatment of GPC3+ malignancies.