

Introduction

MicroRNAs (miRNAs) are a family of naturally-occurring small, non-coding RNAs and serve as small snippets of genetic material that regulate gene expression. As a result, miRNAs modulate a wide range of biological processes including; cell cycle control and apoptosis, cell signaling and differentiation, cell adhesion, and motility. Due to the inherent ability of miRNAs to concurrently target multiple pathways, their therapeutic potential to be used as anti-cancer drugs is attractive.

Currently, we are investigating the mode of action and development of a novel synthetic miRNA 193a-3p mimic (INT-1B3), functioning as a tumor suppressor in variety of cancers, targeting multiple hallmarks of cancer, and representing potential new approach to immunotherapy as our therapeutic candidate.

Concluding Remarks

- Identification of a novel LNP formulation for efficient *in vivo* delivery of functional miRNA in experimental tumors upon systemic administration
- Anti-tumor activity (tumor growth inhibition) demonstrated in human tumor xenograft models
- Pronounced immuno-oncology characteristics, with modulation of immunosuppressive tumor microenvironment and T-cell-dependent long-term immunity against cancer cells
- Selection of INT-1B3 as InteRNA's development candidate for therapeutic intervention in HCC, melanoma and/or TNBC

Future prospective:

- Confirmatory studies in various syngeneic tumor models
- IND-/CTA-enabling activities (CMC and pharmacology)
- IND/CTA preparation

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Results

INT-1B3 mode of actions in *in vitro* settings

Table1. summary of INT-1B3 MoA in *in vitro* settings

Cancer type	Cell line	Viability (96h)	Apoptosis (48h/72h)	Cell cycle arrest (72h)	Motility (18-24h)
Liver	Hep3B, SNU449			G2/M	
Liver	Huh7			-	N.A.
Melanoma	A2058			G2/M	
Lung	A549, H460			SubG1	, -
Lung	H1299			-	N.A.
Lung	H1975			G2/M	N.A.
Breast	4T1			N.A.	
Breast	EMT6			N.A.	-
Pancreas	Panc-1			G2/M	N.A.
Colon	HCT116			-	N.A.

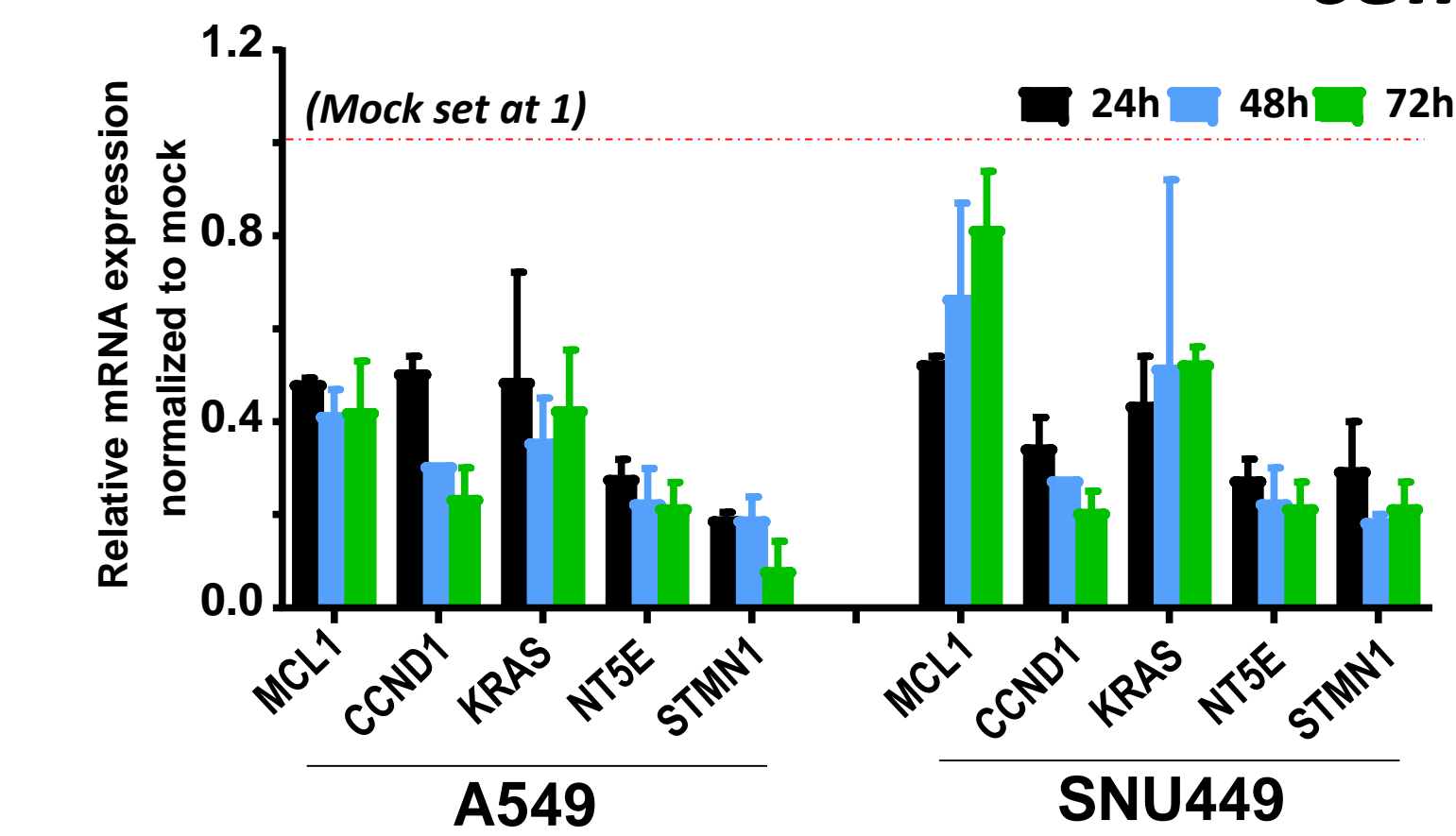
INT-1B3 has been tested in cell-based assays in a variety of cell lines. Different concentrations (1, 3, 10 nM) of INT-1B3 was taken along with proper controls (untreated, mock and scrambled). The 10 nM as ideal concentration for *in vitro* assays are shown in the table. The readouts have been recorded at 24, 48, 72, and 96h post-transfection, results have been normalized to mock.

Assays used:
Viability: MTS or cell count
Cell cycle: Nuclei imaging, Flow cytometry
Apoptosis: Caspase 3/7
Motility: Boyden chamber

|| viability < 50%
| viability > 50%
N.A. (not available)

|| induction > 2x
| induction < 2x

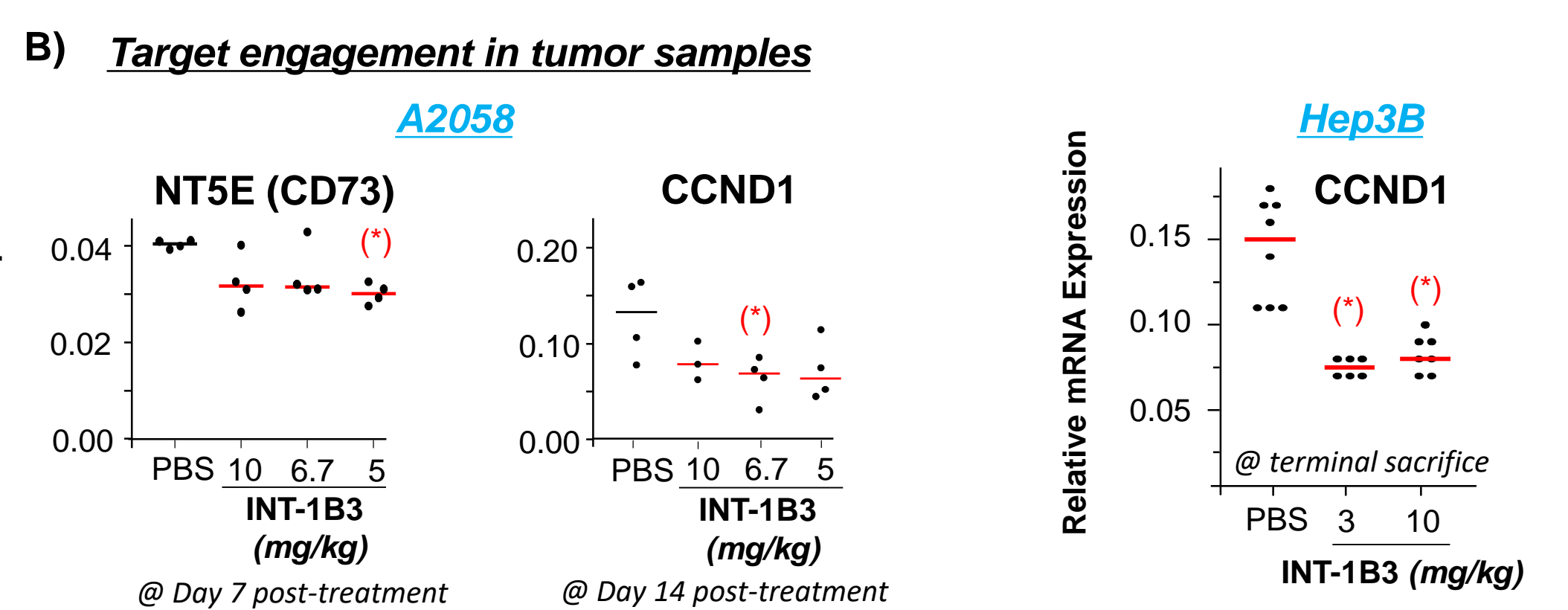
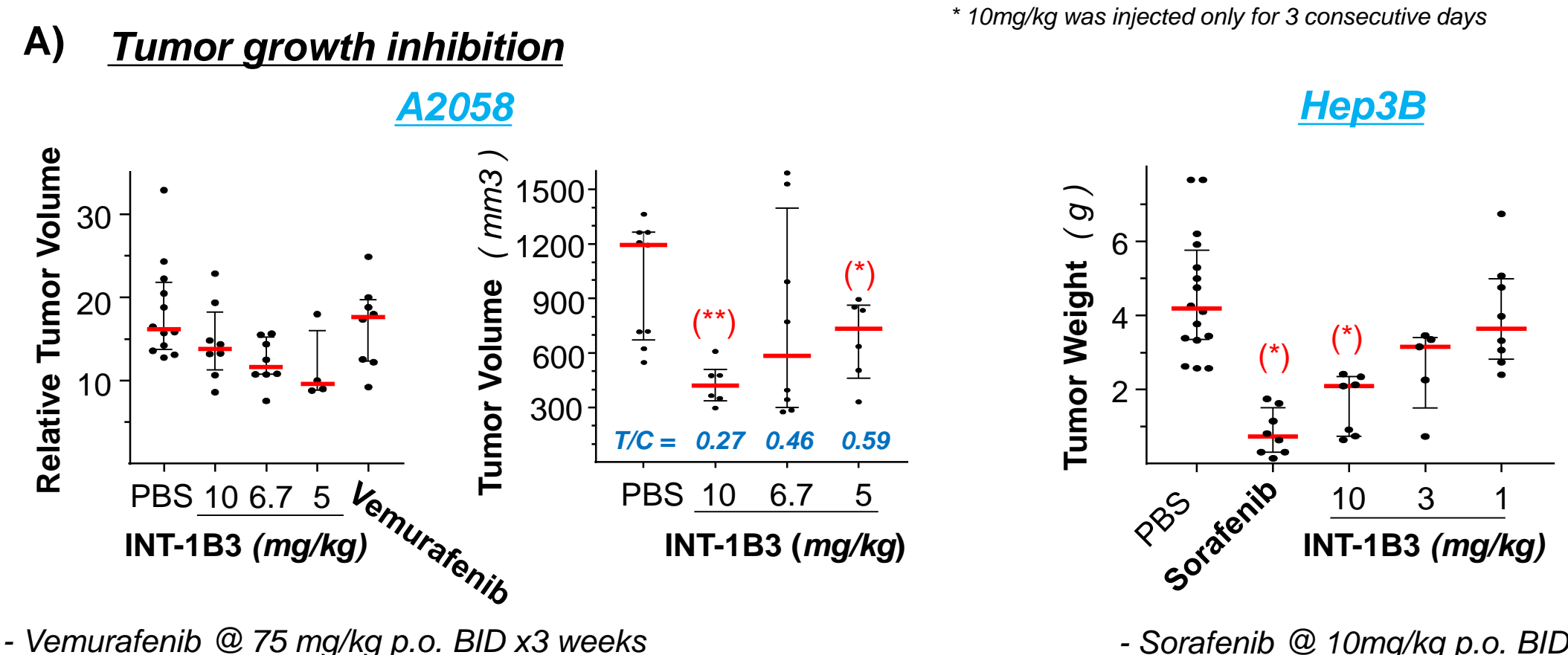
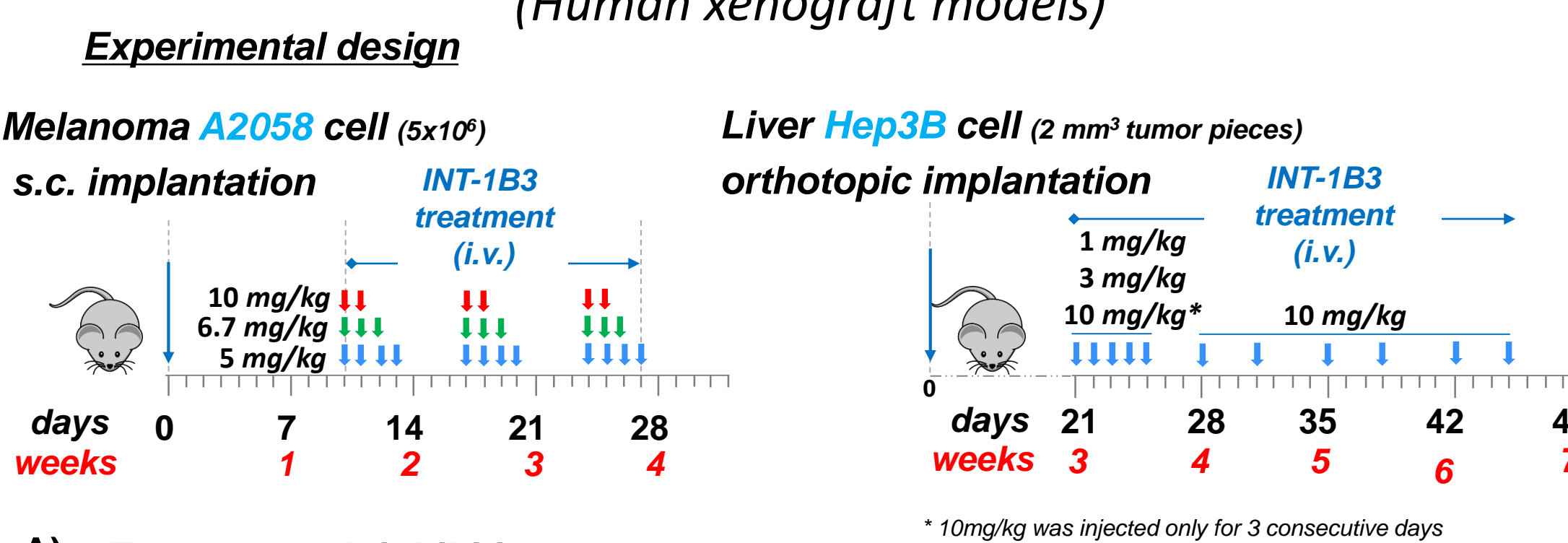
INT-1B3 targets pivotal oncogenes to induce cell death and apoptosis



A vast number of predicted INT-1B3 target genes based on our *in vitro* RNA seq. data and online databases were tested and validated using a variety of cell lines. This graph shows a few examples of oncogenic targets of INT-1B3 that are 50%-90% downregulated compared to Mock control. These targets have also been validated by 3'UTR assay. Different concentration of INT-1B3 (1, 3, 10 nM) and proper controls were tested at different time points (24, 48, and 72h). The 10nM results are shown and results have been normalized to mock.

Cell lines: A549: Lung cancer (NSCLC); SNU449: Liver cancer (HCC). Target genes: MCL1, CCND1, and STMN1: Role in cell cycle and cell proliferation; KRAS: Oncogene; NT5E (CD73): Enzyme involved in adenosine generation pathway.

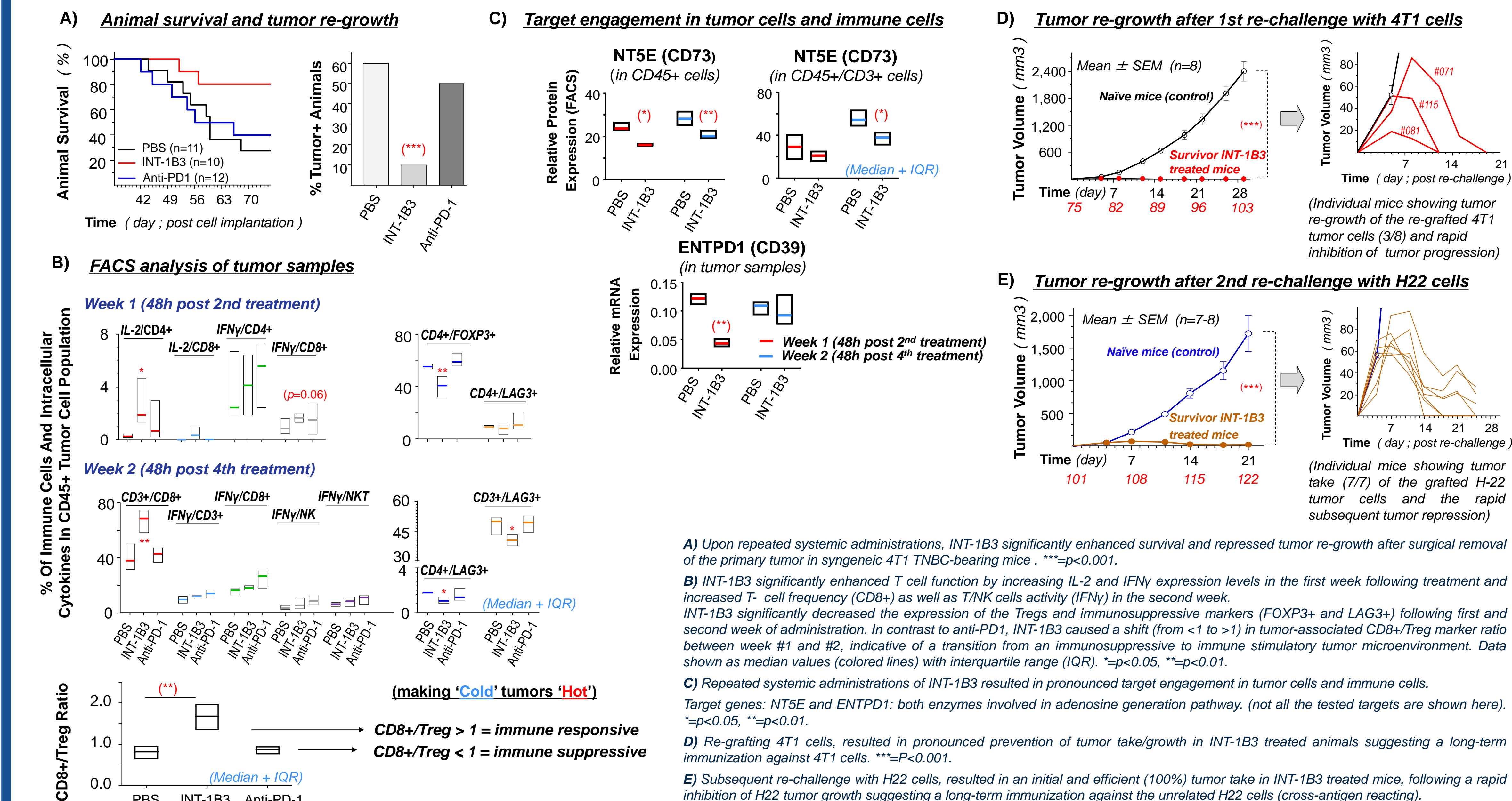
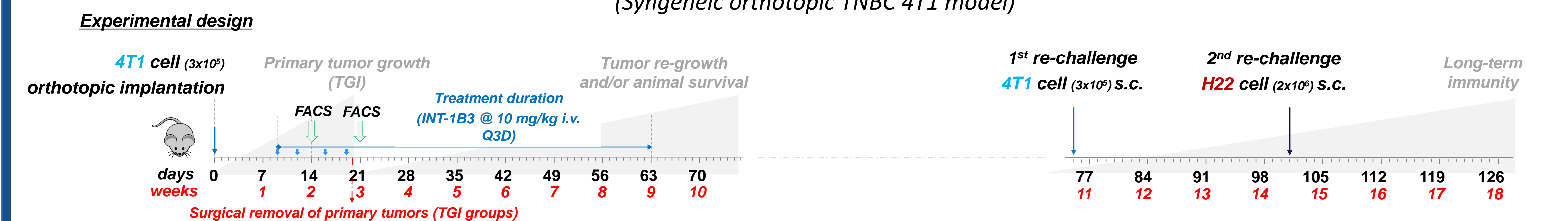
INT-1B3 anti-tumor activity (Human xenograft models)



A) Upon repeated systemic administrations, INT-1B3 significantly inhibited tumor growth in a dose-dependent manner in human melanoma and HCC experimental tumor models. (In the presented graphs each dot indicates an individual tumor sample.) Data shown as median values (red line) with interquartile range. * $p < 0.05$.
▪ In comparison to benchmark drug Vemurafenib, INT-1B3 significantly inhibits tumor growth in B-RAF mutated A2058 melanoma cells.
▪ In comparison to benchmark drug Sorafenib, INT-1B3 dosed at 10 mg/kg / administration inhibits tumor growth in Hep3B HCC cells to a similar extent.

B) Repeated systemic administrations of INT-1B3 result in pronounced target engagement in melanoma and HCC experimental tumor models. (In the presented graphs each dot indicates an individual tumor sample.) Data shown as median values (red line) with interquartile range. * $p < 0.05$.
CCND1: an important cell cycle regulator; NT5E: involved in adenosine generation pathway.
(Different INT-1B3 target genes involved in cell cycle, apoptosis and, immune pathway have been shown to be down-regulated in tumor cells (data not shown here).)

INT-1B3 effect on animal survival and long-term immunity (Syngeneic orthotopic TNBC 4T1 model)



A) Upon repeated systemic administrations, INT-1B3 significantly enhanced survival and repressed tumor re-growth after surgical removal of the primary tumor in syngeneic 4T1 TNBC-bearing mice. ** $p < 0.001$.

B) INT-1B3 significantly enhanced T cell function by increasing IL-2 and IFN γ expression levels in the first week following treatment and increased T_H cell frequency (CD8 $^{+}$) as well as T_H1 cells activity (IFN γ) in the second week. INT-1B3 significantly decreased the expression of the Tregs and immunosuppressive markers (FOXP3 $^{+}$ and LAG3 $^{+}$) following first and second week of administration. In contrast to anti-PD-1, INT-1B3 caused a shift (from <1 to >1) in tumor-associated CD8 $^{+}$ /Treg marker ratio between week #1 and #2, indicative of a transition from an immunosuppressive to immune stimulatory tumor microenvironment. Data shown as median values (colored lines) with interquartile range (IQR). * $p < 0.05$, ** $p < 0.01$.

C) Repeated systemic administrations of INT-1B3 resulted in pronounced target engagement in tumor cells and immune cells. Target genes: NT5E and ENTPD1: both enzymes involved in adenosine generation pathway. (not all the tested targets are shown here). * $p < 0.05$, ** $p < 0.01$.

D) Re-grafting 4T1 cells, resulted in pronounced prevention of tumor take/growth in INT-1B3 treated animals suggesting a long-term immunization against 4T1 cells. *** $p < 0.001$.

E) Subsequent re-challenge with H22 cells, resulted in an initial and efficient (100%) tumor take in INT-1B3 treated mice, following a rapid inhibition of H22 tumor growth suggesting a long-term immunization against the unrelated H22 cells (cross-antigen reacting).