OMTO, Volume *31*

Supplemental information

A novel GPI-anchored dominant-negative TGF-β receptor II renders T cells unresponsive to TGF-β signaling

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Fig.S1: GPI-ecto-TGFβRII does interfere with TGFβ induced SMAD2/3 signalling in ATCs and Jurkat cells

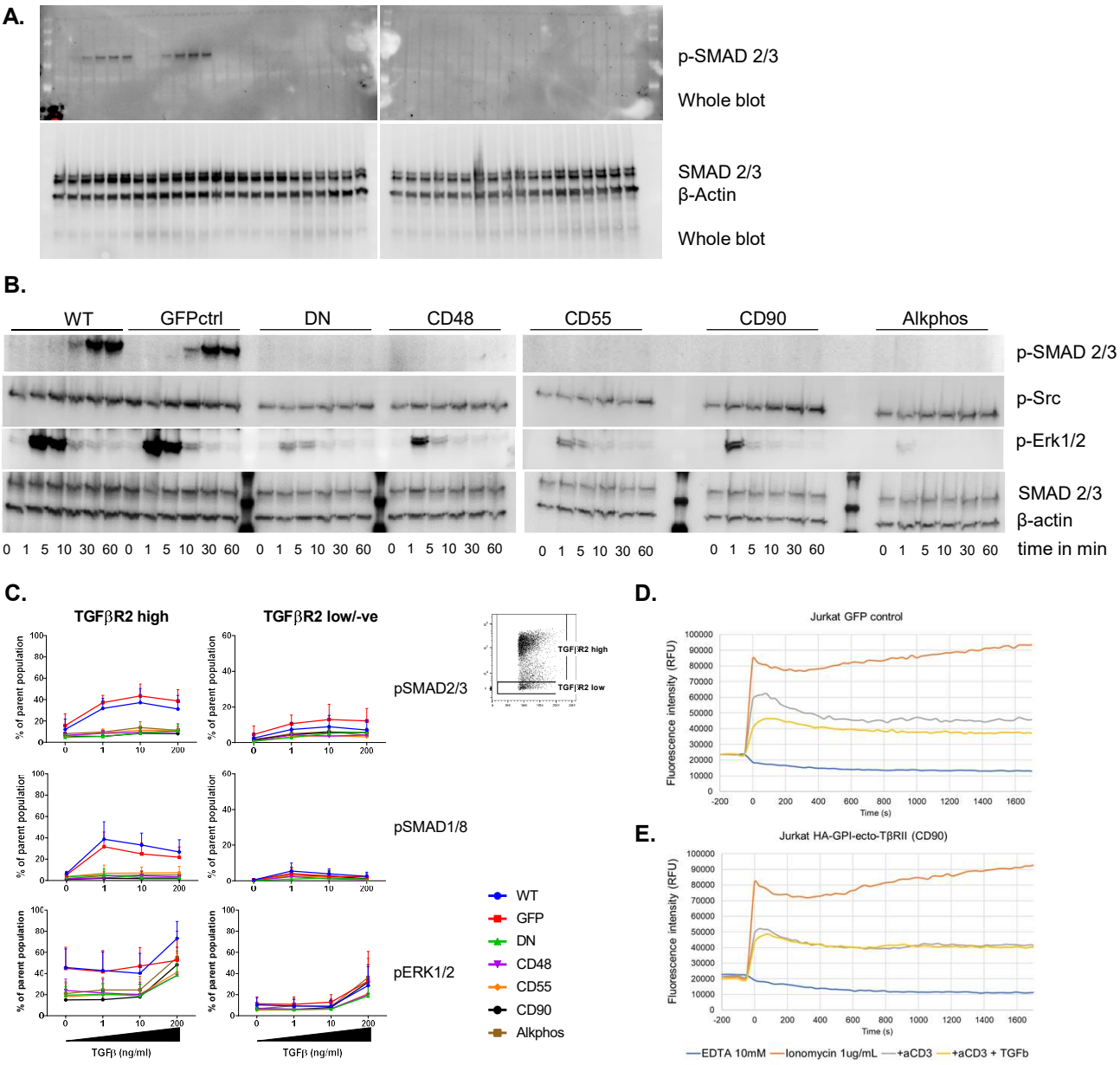


Fig. S1: A) Whole WB of pSMAD2/3 from Fig. 2. B) WB of strongly inhibited TGFβ-dependent SMAD and ERK signalling in Jurkat cells expressing TGFβ-decoy receptors. No changes in Src signaling was observed. C) Flow cytometric analysis of p-SMAD2/3 and 1/8 and ERK1/2 in Jurkat cells gated for either high or low expression of TGFβR2 (decoy or WT). D+E) Readings of calcium flux in the presence of TGFβ in either stimulated GFP-ctrl. or HA-GPI-ecto-TβRII expressing Jurkat cells.

Fig. S2: GPI-ecto-TGFbRII in ATCs does not affect stimulation, exhaustion or differentiation in TGFβ low culture conditions

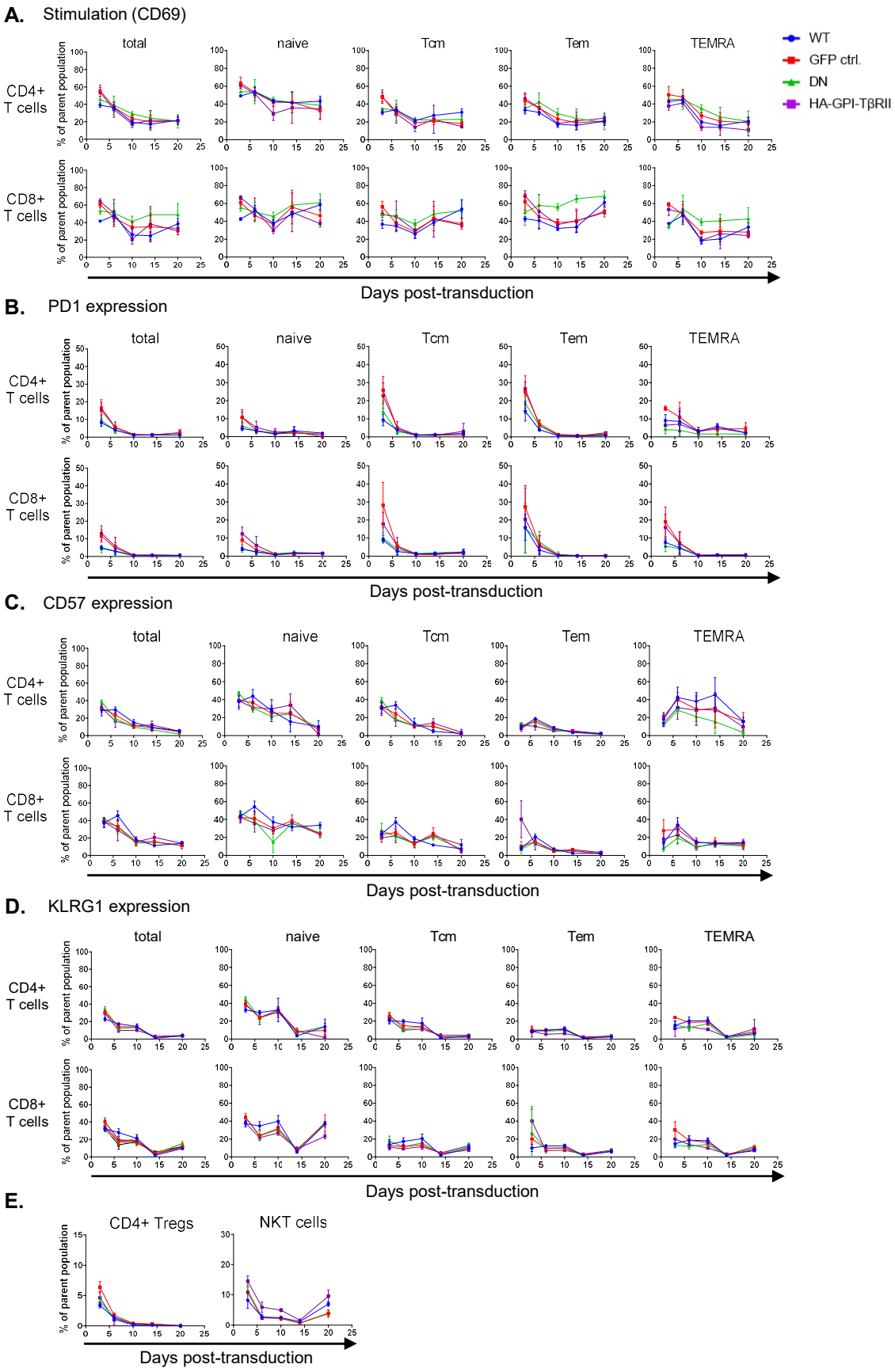


Fig. S2: Expression of TGFβ-decoy receptor does not affect primary T cell marker expression for stimulation, exhaustion or differentiation per se. A) Line graphs representing the expression of CD69 as a marker for T cell stimulation, B) PD1 as a marker for exhaustion, and C) CD57 and D) KLRG1 as marker for T cell differentiation. E) the ratio of CD4+ T cells as well as NKT cells is not affected either. Line graphs display the mean and SEM of at least 6 biological replicates.

Fig. S3: HA-GPI-ecto-TGFbRII has no effect on stimulation or exhaustion or the expression of the chosen marker for differentiation in ATCs in the presence of recombinant TGFb

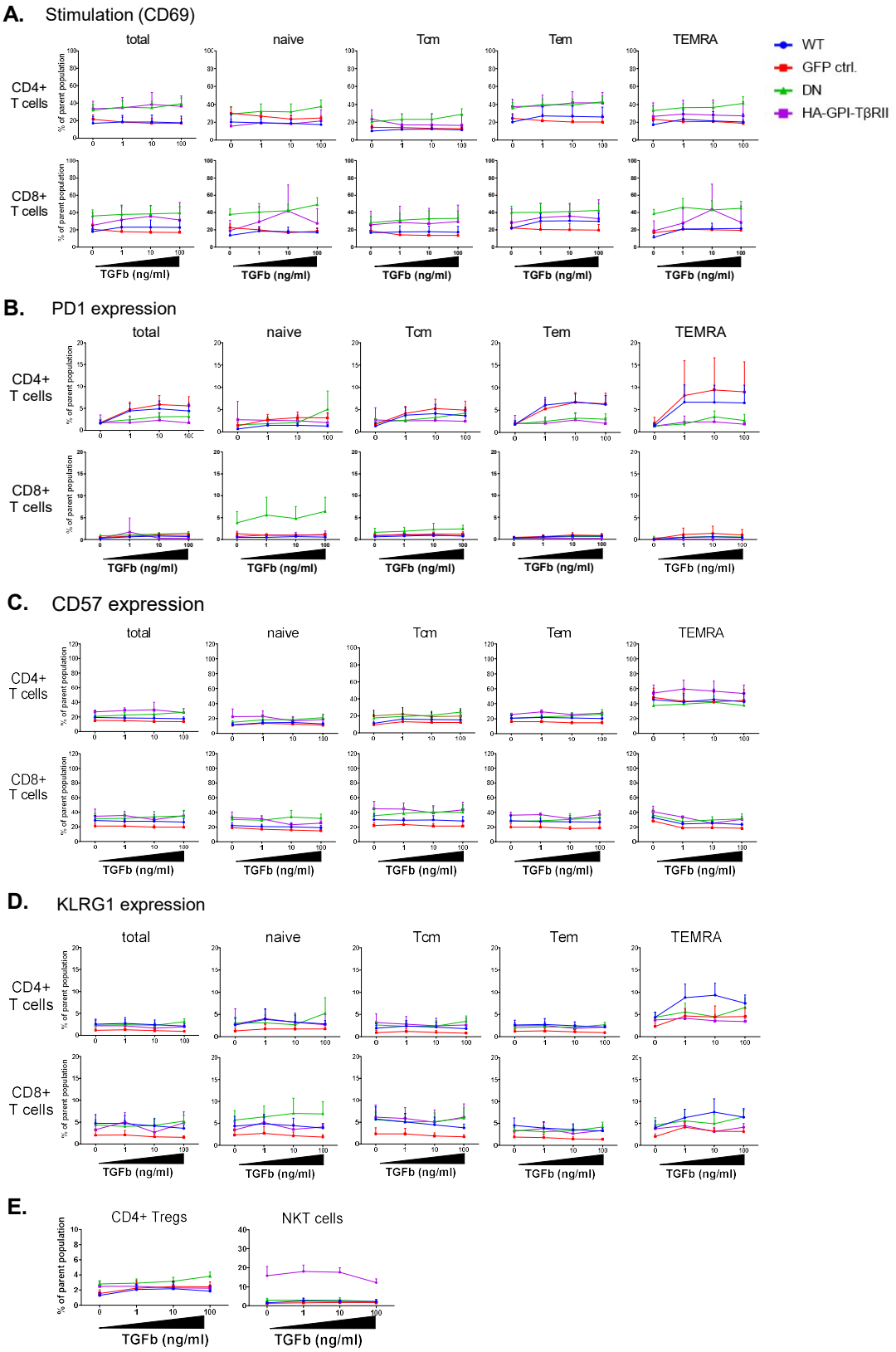
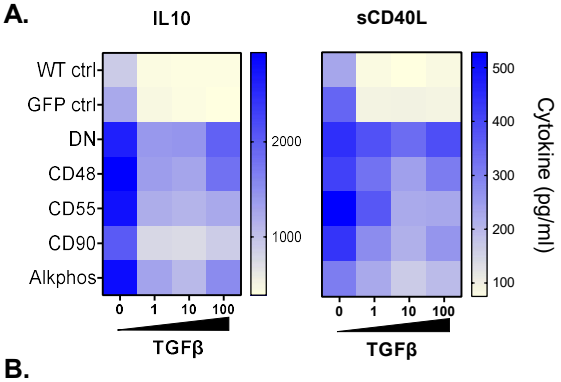


Fig. S3: Expression of TGFβ-decoy receptor does not affect primary T cell marker expression for stimulation, exhaustion or differentiation in the presence of different concentrations of TGFβ. A) Line graphs representing the expression of CD69 as a marker for T cell stimulation, B) PD1 as a marker for exhaustion, and C) CD57 and D) KLRG1 as marker for T cell differentiation. E) the ratio of CD4+ T cells is not affected whereas the proliferation of NKT cells seems slightly enhanced in cells expressing HA-GPI-ecto-TβRII. Line graphs display the mean and SEM of at least 6 biological replicates.

Fig S4: HA-GPI-ecto-TβII transduced ATCs maintain cytokine production in the presence of TGFb

A.



B.

IFNg		GFP vs HA-DN		GFP vs HA-Alkphos	
	TGFb conc.	p value	significance	p value	significance
	untreated	0.07213	NA	0.08900	NA
	1ng/ml	0.00158	**	0.00255	**
	10ng/ml	0.00350	**	0.00631	**
	100ng/ml	0.00092	***	0.00649	**

GM-CSF		GFP vs HA-DN		GFP vs HA-Alkphos	
	TGFb conc.	p value	significance	p value	significance
	untreated	0.05978	NA	0.36468	NA
	1ng/ml	0.00026	***	0.00024	***
	10ng/ml	0.00014	***	0.00034	***
	100ng/ml	0.00027	***	0.00196	**

TNFα		GFP vs HA-DN		GFP vs HA-Alkphos	
	TGFb conc.	p value	significance	p value	significance
	untreated	0.009872	**	0.07086	NA
	1ng/ml	0.000855	***	0.00287	**
	10ng/ml	0.000821	***	0.00661	**
	100ng/ml	0.000332	***	0.00736	**

TNFβ		GFP vs HA-DN		GFP vs HA-Alkphos	
	TGFb conc.	p value	significance	p value	significance
	untreated	0.329954	NA	0.7425	NA
	1ng/ml	0.089237	NA	0.2067	NA
	10ng/ml	0.084746	NA	0.2439	NA
	100ng/ml	0.042144	*	0.1323	NA

MIP-1b		GFP vs HA-DN		GFP vs HA-Alkphos	
	TGFb conc.	p value	significance	p value	significance
	untreated	0.521679	NA	0.98554	NA
	1ng/ml	0.036379	*	0.07303	NA
	10ng/ml	0.050498	NA	0.04275	*
	100ng/ml	0.018459	*	0.05823	NA

IL3		GFP vs HA-DN		GFP vs HA-Alkphos	
	TGFb conc.	p value	significance	p value	significance
	untreated	0.02701	*	0.62017	NA
	1ng/ml	0.00253	**	0.02117	*
	10ng/ml	0.00184	**	0.04388	*
	100ng/ml	0.00127	**	0.03139	*

IL4		GFP vs HA-DN		GFP vs HA-Alkphos	
	TGFb conc.	p value	significance	p value	significance
	untreated	0.20879	*	0.66077	NA
	1ng/ml	0.00367	**	0.01860	*
	10ng/ml	0.02040	*	0.03725	*
	100ng/ml	0.00061	***	0.01556	*

IL5		GFP vs HA-DN		GFP vs HA-Alkphos	
	TGFb conc.	p value	significance	p value	significance
	untreated	0.52152	NA	0.37745	NA
	1ng/ml	0.03666	*	0.04560	*
	10ng/ml	0.06233	NA	0.04387	*
	100ng/ml	0.03469	*	0.04952	*

IL6		GFP vs HA-DN		GFP vs HA-Alkphos	
	TGFb conc.	p value	significance	p value	significance
	untreated	0.13642	NA	0.54948	NA
	1ng/ml	0.00010	***	0.05073	NA
	10ng/ml	0.00001	***	0.00791	**
	100ng/ml	0.00334	**	0.00378	**

IL13		GFP vs HA-DN		GFP vs HA-Alkphos	
	TGFb conc.	p value	significance	p value	significance
	untreated	0.98495	NA	0.79050	NA
	1ng/ml	0.00016	***	0.00049	***
	10ng/ml	0.00023	***	0.00205	**
	100ng/ml	0.00013	***	0.00509	**

IL10		GFP vs HA-DN		GFP vs HA-Alkphos	
	TGFb conc.	p value	significance	p value	significance
	untreated	0.00498	**	0.02654	*
	1ng/ml	0.00097	***	0.01471	*
	10ng/ml	0.00032	***	0.02407	*
	100ng/ml	0.00019	***	0.00593	**

sCD40L		GFP vs HA-DN		GFP vs HA-Alkphos	
	TGFb conc.	p value	significance	p value	significance
	untreated	0.29442	NA	0.58031	NA
	1ng/ml	0.00146	**	0.00138	**
	10ng/ml	0.00171	**	0.03216	*
	100ng/ml	0.00094	***	0.00906	**

Fig. S4A) Heat map presentation of concentrations of IL10 and sCD40L measured in cell culture supernatants after ATC culture in the presence of different concentrations of TGFβ. B) Statistical analyses of cytokine concentrations measured in culture supernatants of GFP ctrl. and TGFβ-decoy receptor expressing ATCs. Heat maps display the data of 6 biological replicates.