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Supplemental Information

Regulated Expansion and Survival of Chimeric Antigen Receptor-Modified T Cells Using Small Molecule-Dependent Inducible MyD88/CD40

Aaron E. Foster, Aruna Mahendravada, Nicholas P. Shinners, Wei-Chun Chang, Jeannette Crisostomo, An Lu, Mariam Khalil, Eva Morschl, Joanne L. Shaw, Sunandan Saha, MyLinh T. Duong, Matthew R. Collinson-Pautz, David L. Torres, Tania Rodriguez, Tsvetelina Pentcheva-Hoang, J. Henri Bayle, Kevin M. Slawin, and David M. Spencer

SUPPLEMENTAL TABLES

Supplemental Table 1. Fold-change in cytokine production following iMC activation with rimiducid.

Cytokine production (fold-increase)				
Induced			**Non-induced**	
GM-CSF	2.0		IL-2	1.1
IFN-γ	3.4		IL-4	1.4
IL-13	4.6		IL-7	1.3
IL-5	2.4		IL-10	1.2
IL-8	2.8		IL-12p70	1.3
IP-10	5.8		IL-15	0.9
TNF-α	2.6		IL-17	1.1

Calculated as fold-increase following 10 nM rimiducid stimulation compared to iMC-modified T cells cultured in media alone.

Supplemental Table 2. Gene ontology pathway analysis.

Name	P-value
Apoptosis GenMAPP	5.41E-08
Apoptosis	7.14E-08
Eicosanoid synthesis	0.0004
Cholesterol biosynthesis	0.002
Inflammatory response pathway	0.002
Small ligand GPCRs	0.002
Apoptosis KEGG	0.003
Nuclear receptors	0.004
Hypertrophy model	0.004
S1P signaling	0.006
Smooth muscle contraction	0.01
MAPK cascade	0.03

Supplemental Table 3. KEGG pathway gene enrichment signature.

Gene Set Name	# Genes in Gene Set (K)	# Genes in Overlap (k)	k/K	p-value	FDR q-value
KEGG_SMALL_CELL_LUNG_CANCER	84	10	0.119	1.52E-10	2.82E-08
KEGG_PATHWAYS_IN_CANCER	328	16	0.0488	4.04E-10	3.76E-08
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	267	13	0.0487	1.85E-08	1.15E-06
KEGG_MAPK_SIGNALING_PATHWAY	267	11	0.0412	1.21E-06	5.64E-05
KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY	102	7	0.0686	4.01E-06	1.49E-04
KEGG_JAK_STAT_SIGNALING_PATHWAY	155	8	0.0516	6.87E-06	2.13E-04
KEGG_APOPTOSIS	88	6	0.0682	2.07E-05	5.49E-04
KEGG_FOCAL_ADHESION	201	8	0.0398	4.47E-05	9.48E-04
KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY	62	5	0.0806	4.59E-05	9.48E-04
KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY	108	6	0.0556	6.57E-05	1.22E-03

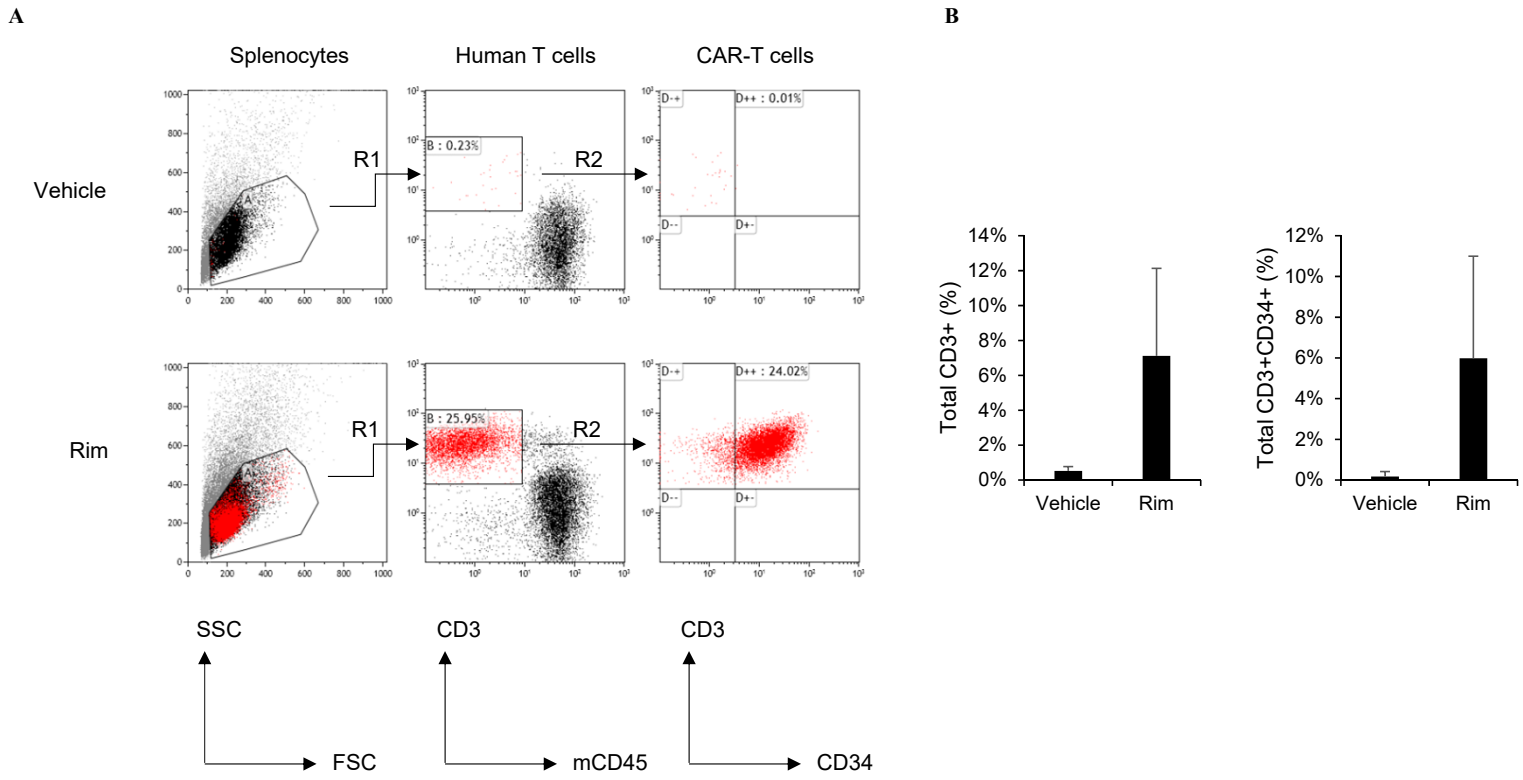
Supplemental Table 4. Transcription factor target gene enrichment signature.

Gene Set Name	# Genes in Gene Set (K)	# Genes in Overlap (k)	k/K	p-value	FDR q-value
GGGCGGR_V$SP1_Q6	2940	64	0.0218	1.56E-18	9.57E-16
V$NFKB_Q6	254	19	0.0748	4.43E-15	1.36E-12
CAGGTG_V$E12_Q6	2485	52	0.0209	2.17E-14	4.45E-12
V$NFKAPPAB_01	251	17	0.0677	6.32E-13	9.72E-11
GGGAGGRR_V$MAZ_Q6	2274	45	0.0198	1.02E-11	1.06E-09
V$CREL_01	256	16	0.0625	1.03E-11	1.06E-09
V$NFKAPPAB65_01	237	15	0.0633	3.86E-11	3.39E-09
RYTTCCTG_V$ETS2_B	1085	29	0.0267	8.17E-11	6.28E-09
RTAAACA_V$FREAC2_01	919	25	0.0272	1.23E-09	7.76E-08
TGGAAA_V$NFAT_Q4_01	1896	37	0.0195	1.26E-09	7.76E-08

Supplemental Table 5. Immunological gene enrichment signature.

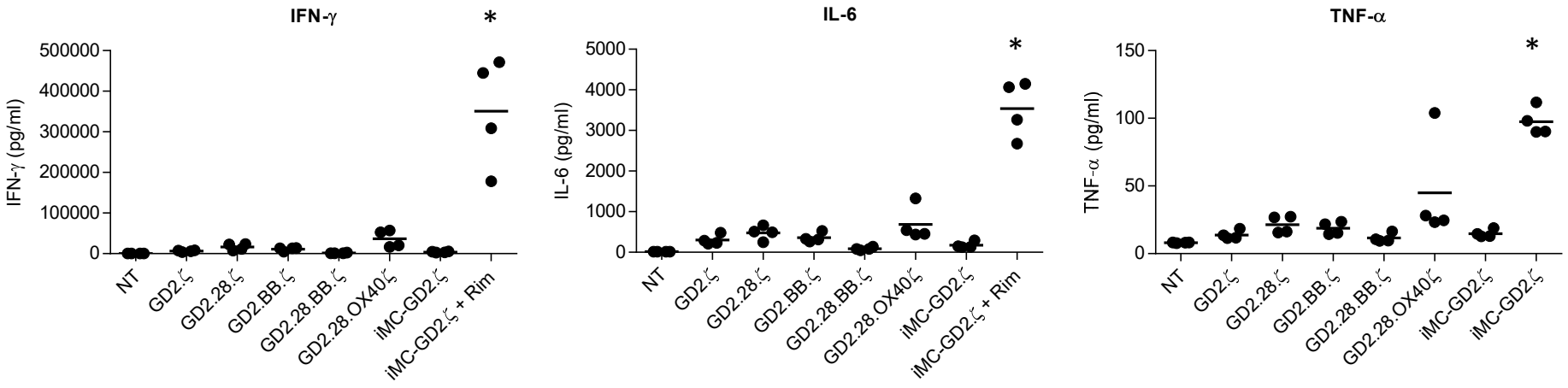
Gene Set Name	# Genes in Gene Set (K)	# Genes in Overlap (k)	k/K	p-value	FDR q-value
GSE2706_UNSTIM_VS_2H_LPS_DC_DN	200	35	0.175	6.09E-40	1.16E-36
GSE9988_LOW_LPS_VS_CTRL_TREATED_MONOCYTE_UP	200	34	0.17	2.29E-38	2.19E-35
GSE2706_UNSTIM_VS_2H_LPS_AND_R848_DC_DN	200	33	0.165	8.31E-37	5.29E-34
GSE2706_UNSTIM_VS_8H_R848_DC_DN	200	30	0.15	3.10E-32	9.87E-30
GSE9988_ANTI_TREM1_VS_ANTI_TREM1_AND_LPS_MONOCYTE_DN	200	30	0.15	3.10E-32	9.87E-30
GSE9988_ANTI_TREM1_VS_LOW_LPS_MONOCYTE_DN	200	30	0.15	3.10E-32	9.87E-30
GSE2706_UNSTIM_VS_2H_R848_DC_DN	200	29	0.145	9.53E-31	2.28E-28
GSE9988_LOW_LPS_VS_VEHICLE_TREATED_MONOCYTE_UP	200	29	0.145	9.53E-31	2.28E-28
GSE22886_CTRL_VS_LPS_24H_DC_DN	200	28	0.14	2.81E-29	4.47E-27
GSE9988_ANTI_TREM1_VS_LPS_MONOCYTE_DN	200	28	0.14	2.81E-29	4.47E-27

Figure S1



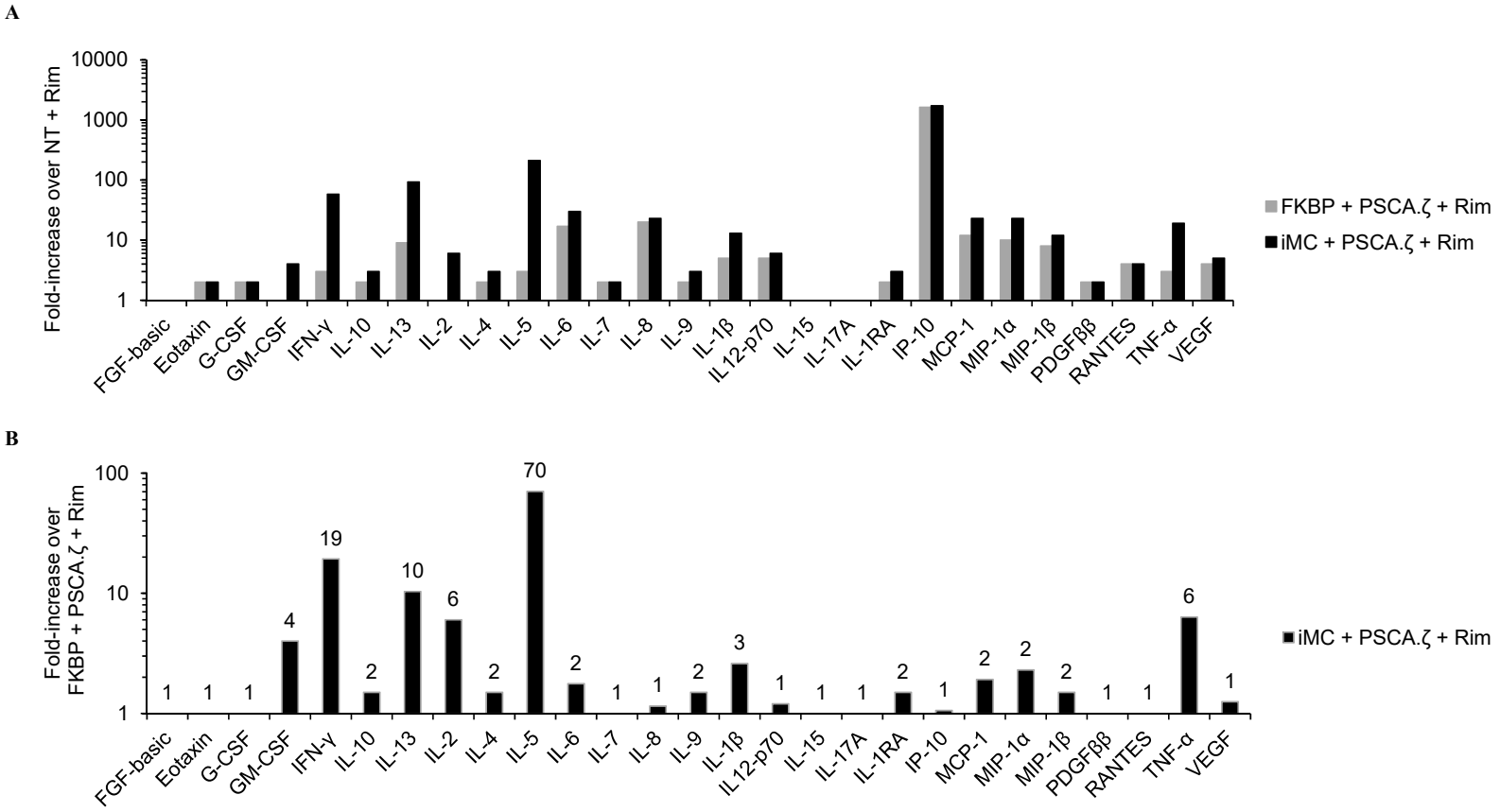
Supplemental Figure 1. Activation of iMC increases the frequency of CAR-T cells following Rim administration. A) NSG mice (n = 5 per group) engrafted with HPAC tumors were treated with 1.0 x 10^6 iMC-PSCA.ζ-modified T cells and were subsequently injected i.p. with 5 mg/kg Rim on a weekly basis. On day 21 splenocytes were harvested and analyzed by flow cytometry. Human T cells were detected using a "live cell" region (R1) by gating on forward (FSC) and side scatter (SSC) profiles, followed by gating on a human $CD3^+$/mouse $CD45^-$ region (R2). CAR-T cells were subsequently analyzed using a R1+R2 boolean gate and analyzing for $CD3^+CD34^+$ T cells. **B)** Total percent human T cells for vehicle and Rim-treated mice and total percent gated $CD3^+CD34^+$ CAR-T cells were quantitated.

Figure S2



Supplemental Figure 2. Cytokine production comparison of iMC-GD2.ζ-modified T cells compared to other CAR constructs after coculture with GD2$^+$ HT-144 tumor cells. Non-transduced (NT) T cell or T cells transduced (n = 4) with various GD2-targeted vectors (Figure 7) were cocultured with HT-144 tumor cells at an effector to target (E:T) of 1:10 and analyzed for IL-6, IFN-γ and TNF-α production after 48 hours in culture. * indicates a *P*-value <0.001.

Figure S3



Supplemental Figure 3. iMC enhances a broad cytokine production by PSCA-targeted CAR-T cells after stimulation with PSCA$^+$ HPAC tumor cells. Non-transduced T cells and T cells co-transduced with either FKBP-ΔCD19 (FKBP; lacking the MyD88/CD40 signaling domains) and a first generation PSCA.ζ CAR, or transduced with iMC-ΔCD19 (iMC) and the PSCA.ζ CAR were cocultured with Capan-1 tumor cells at an effector to target (E:T) ratio of 1:1 in the presence of 10 nM Rim. After 48 hours, supernatants were analyzed using a 27-plex cytokine/chemokine array. **A)** FKBP + CAR + Rim and iMC + CAR + Rim conditions (two individual donors) were compared to NT + Rim cocultures and a fold-increase was calculated. **B)** Fold-increase of iMC + CAR + Rim was made against FKBP + CAR + Rim to assess the influence of iMC activation on cytokine production during CAR engagement of the PSCA antigen.