Supporting Information

Synthetic matrix scaffolds engineer the in vivo tumor immune microenvironment for immunotherapy screening

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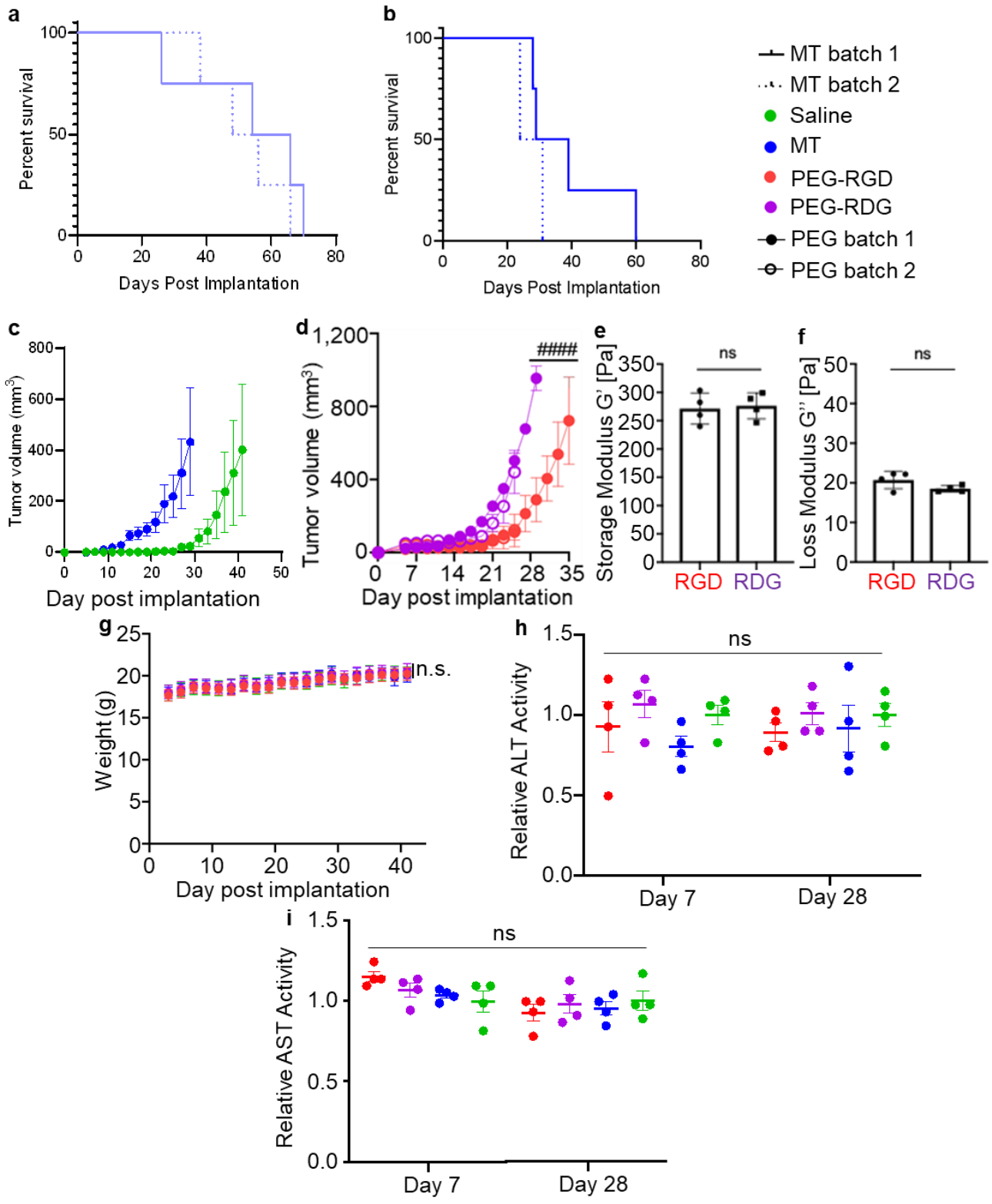


Figure S1: Survival and tumor growth in MFP of immunocompetent animals. Survival of animals after implantation of 250,000 Py230 cells **(a)** and 50,000 E0771 cells **(b)** in different batches of MT into the MFP of C57Bl6 mice. n= 4 animals per group. **(c)** Growth curves of 50,000 E0771 cells implanted in saline and MT into the MFP of C57Bl6 mice. Data

represent data represents mean ± s.e.m. of n=4 animals implanted with one tumor. **(d)** Growth curves of 50,000 E0771 cells implanted into the MFP of C57Bl6 mice in one of two different batches of PEG hydrogel functionalized with either RGD or RDG. Data represent mean ± s.e.m. of n=4 animals implanted with one tumor. **(e-f)** Rheology of PEG hydrogels, including storage (e) and loss modulus (f). **(g)** Animal weight throughout tumor growth after implantation of 50,000 E0771 cells in saline, Matrigel, PEG-RGD, or PEG-RDG. Data represent mean ± s.e.m. of n=16 animals implanted with one tumor. **(h-i)** Relative alanine aminotransferase (h) and aspartate aminotransferase (i) activity in spleens of animals bearing day 7 or day 28 tumors implanted in saline, Matrigel, PEG-RGD, or PEG-RDG. # indicates significance by RM ANOVA with Tukey's post-hoc test; #### indicates $p<0.001$; ns, not significant

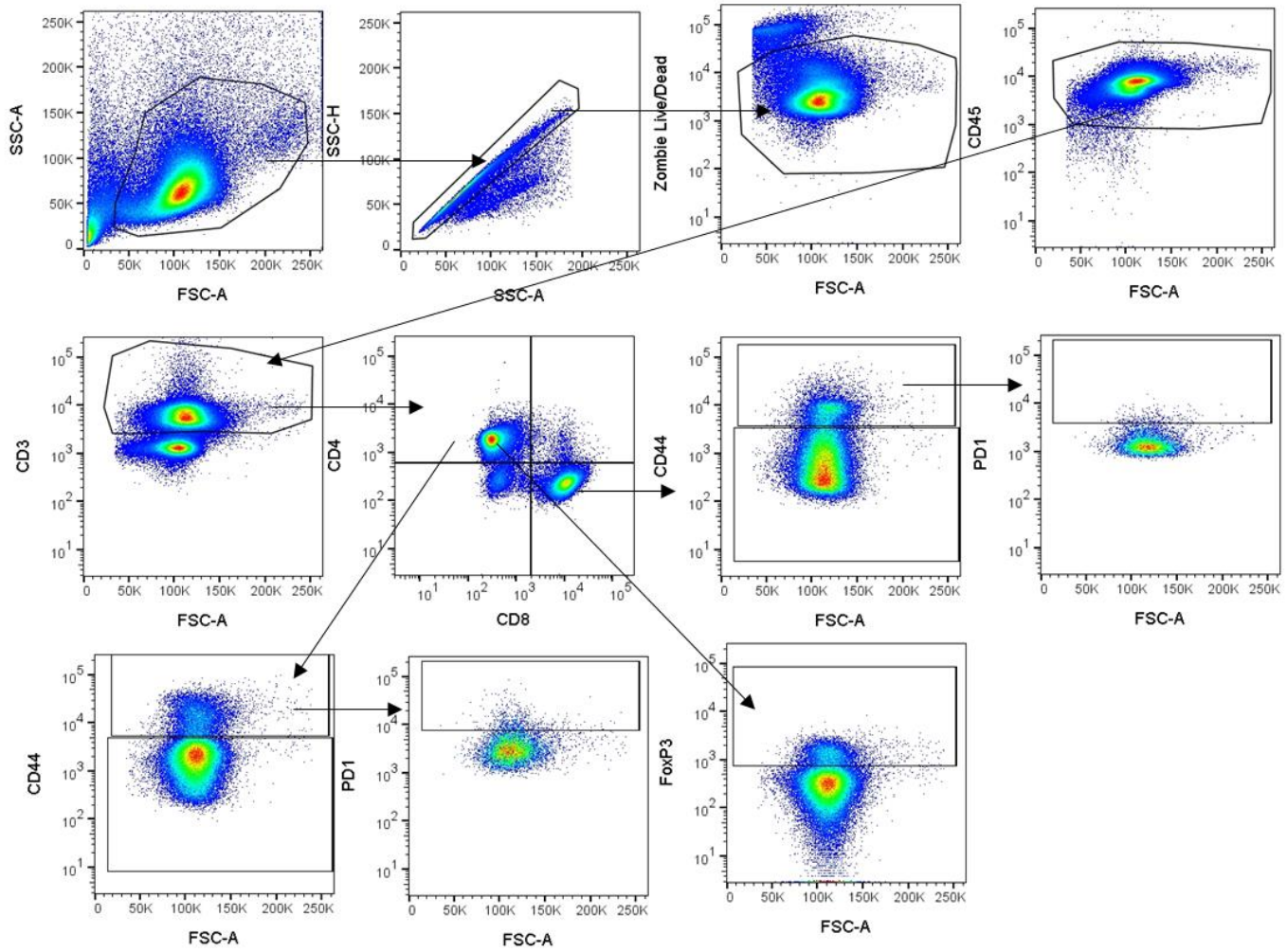


Figure S2: Gating strategy for T cell panel, shown in representative lymph node sample.

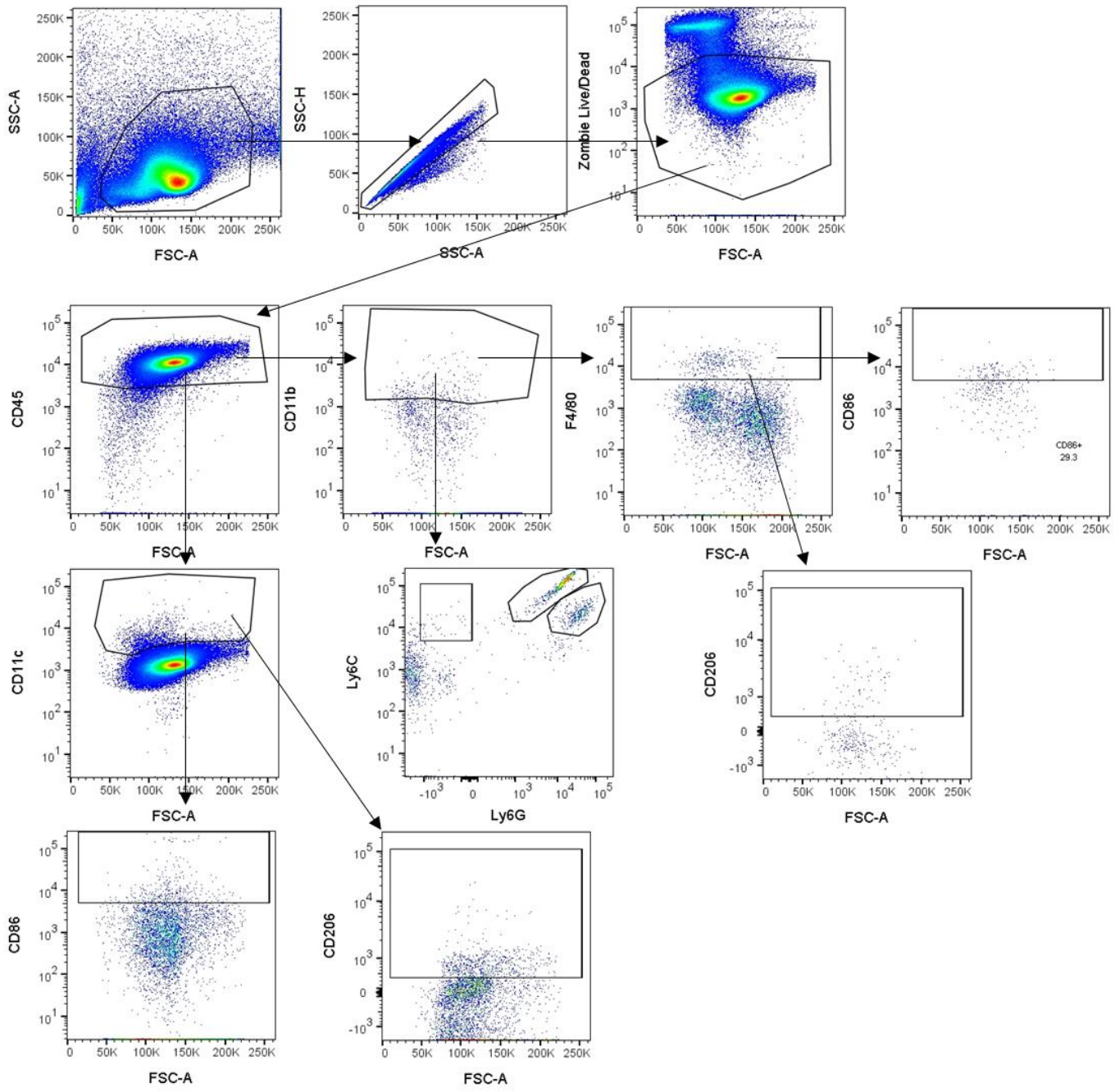
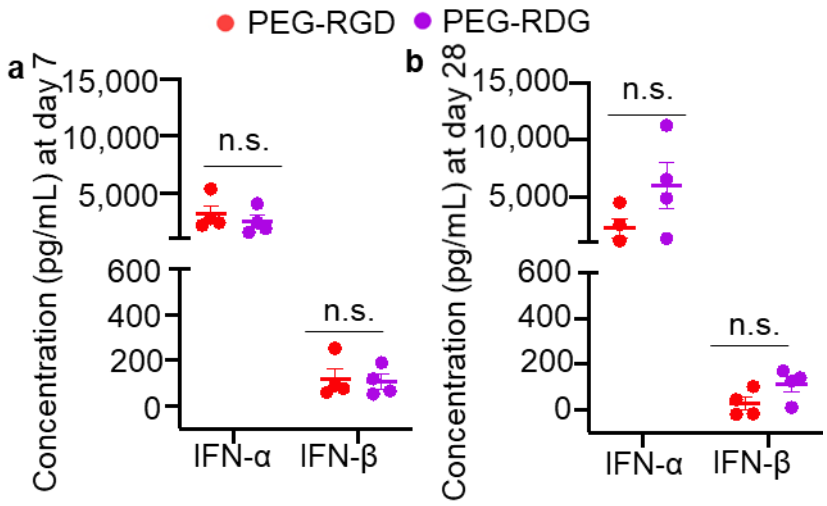


Figure S3: Gating strategy for antigen-presenting/myeloid cell panel, shown in representative lymph node sample.

		day 2		day 7		day 28	
		p-value	significance	p-value	significance	p-value	significance
Inflammatory	IFNg	0.0287	*	0.8809		0.998	
	TNFa	0.0366	*	0.1812		0.996	
	GMCSF	>0.9999		>0.9999		>0.9999	
	IL1b	>0.9999		>0.9999		>0.9999	
	IL6	0.9999		0.0001	***	0.3863	
	IL9	>0.9999		0.9971		>0.9999	
	IL3	0.9996		>0.9999		>0.9999	
	IL12p40	0.9992		0.9905		>0.9999	
	IL12p70	0.9997		0.9997		>0.9999	
Suppressive	IL1a	<0.0001	****	>0.9999		>0.9999	
	IL2	0.9309		0.9719		>0.9999	
	IL4	<0.0001	****	0.459		>0.9999	
	IL5	>0.9999		>0.9999		0.6536	
	IL10	<0.0001	****	>0.9999		>0.9999	
	IL13	0.0102	*	0.0016	***	>0.9999	
	IL15	>0.9999		0.9865		>0.9999	
Growth Factors	GCSF	>0.9999		0.9692		0.9992	
	LIF	>0.9999		0.9999		>0.9999	
	MCSF	0.7044		0.9919		0.9617	
	VEGF	0.0002	***	0.9965		0.9996	
	IL17	>0.9999		>0.9999		>0.9999	
Chemokines	CCL2	>0.9999		>0.9999		>0.9999	
	CCL3	>0.9999		>0.9999		>0.9999	
	CCL4	>0.9999		>0.9999		0.999	
	CXCL2	0.9694		>0.9999		>0.9999	
	CXCL5	0.9985		0.9973		>0.9999	
	CXCL9	0.0453	*	0.9978		0.0394	*
	Eotaxin	0.9996		>0.9999		>0.9999	
	IP10	<0.0001	****	0.9653		>0.9999	
	KC	0.1191		0.0419	*	>0.9999	
	RANTES	<0.0001	****	0.0056	**	0.2145	

Table S1: Statistics between tumors formed within PEG-RGD and PEG-RDG hydrogel matrix vehicles in Figure 4g by mixed-effects model with Tukey's post-hoc test. * indicates $p<0.05$, ** indicates $p<0.01$, *** indicates $p<0.005$, **** indicates $p<0.001$, no notation indicates $p>0.05$.



Supplemental Figure 4: Tumor type I interferon levels. **(a-b)** Concentration of IFN-α and IFN-β in tumors at day 7 (a) and day 28 (b) after implantation of 50,000 E0771 cells in PEG-RGD or PEG-RDG matrix scaffolds. Statistical analysis by one-way ANOVA with Tukey's post-hoc comparison. n.s., not significant.

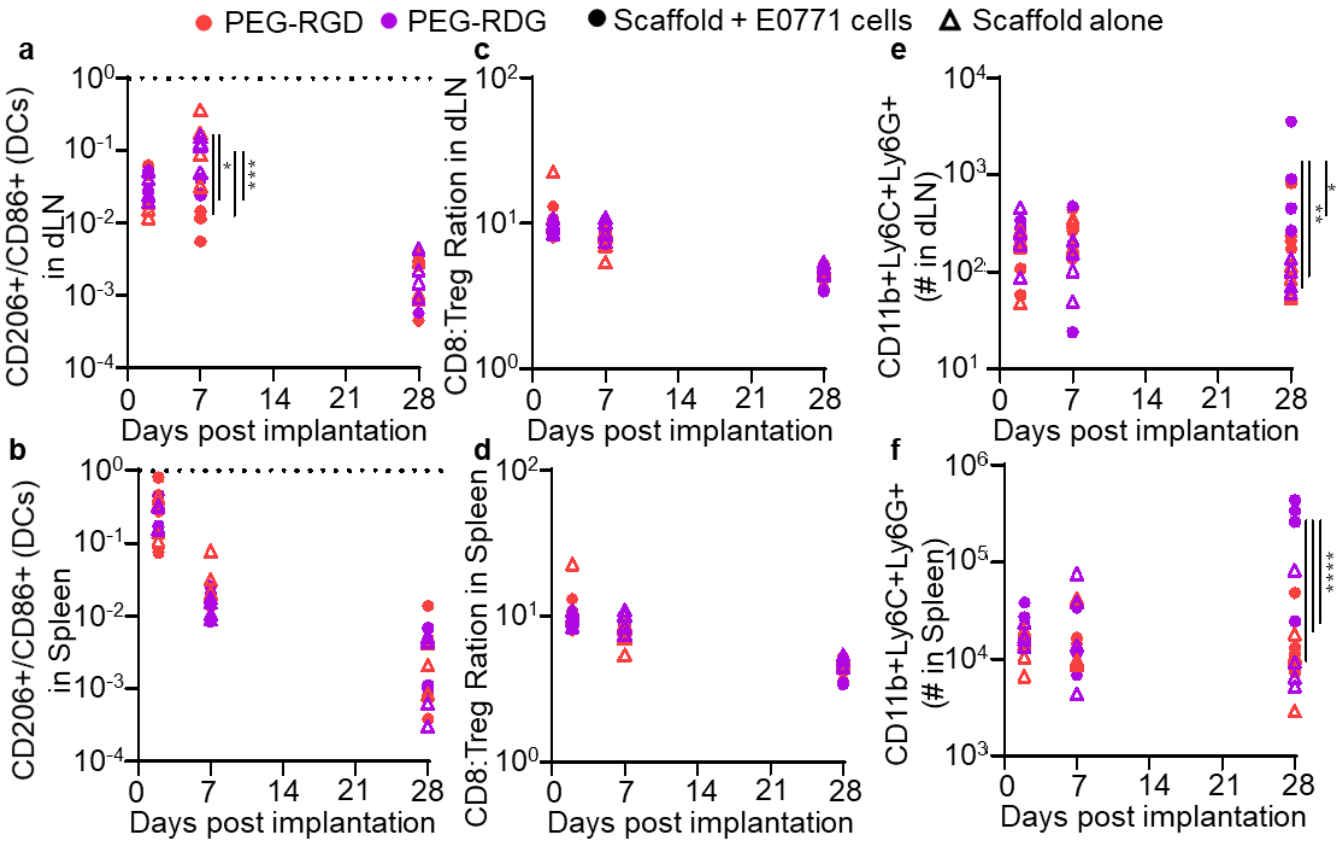


Figure S5: Immune remodeling in lymphoid tissues with scaffold implantation.

CD206+/CD86+ ratio of CD11c+ cells in draining lymph node [dLN] **(a)** and spleen **(b)**; ratio

of CD8+ T cells to CD4+ Tregs in dLN **(c)** and spleen **(d)**; and number of CD11b+Ly6C+Ly6G+ cells in dLN **(e)** and spleen **(f)** after implantation of 50,000 E0771 cells in the MFP of C57Bl6 mice in the indicated matrix vehicle. Each data point represents one tumor implanted animal. * indicates significance by two-way ANOVA with Tukey's post-hoc comparison; n=4 mice. * indicates $p<0.05$, ** indicates $p<0.01$, *** indicates $p<0.005$, **** indicates $p<0.001$.

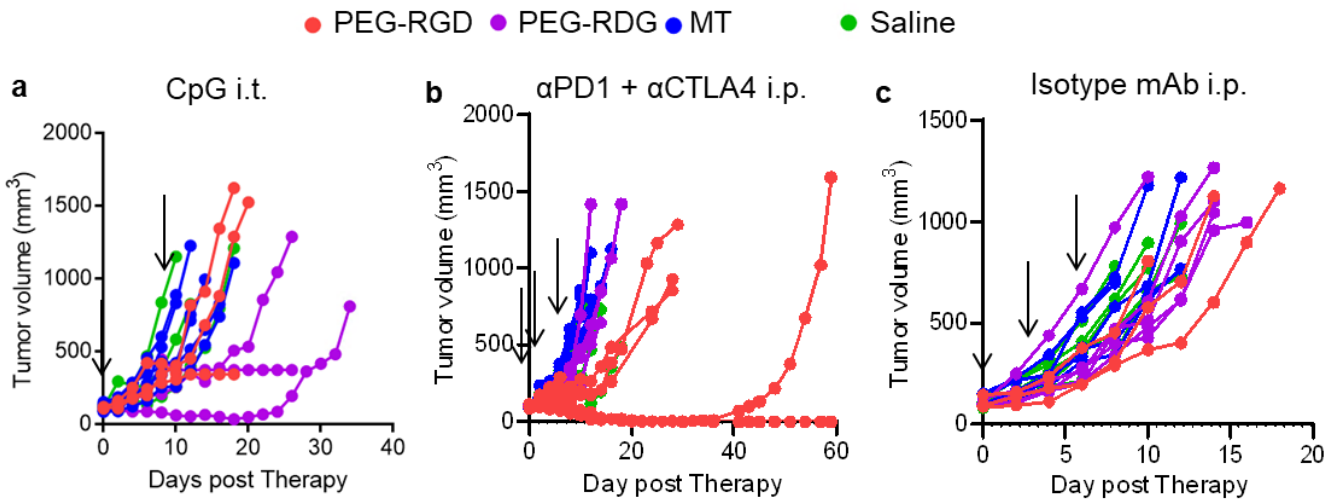


Figure S6: Individual growth curves after therapy: CpG vaccine administered i.t. on day 0 and 7 **(a)**, combination αCTLA4 and αPD1 mAb treatment administered i.p. on days 0, 3, and 6 **(b)**, and isotype control mAb administered i.p. on days 0, 3, and 6 **(c)**. Tumors were formed from 50,000 E0771 cells implanted into C57Bl6 mice within the indicated matrix vehicle, with d0 signifying the first day of treatment (when tumors reached 100 mm^3). n=5-7 per group. Arrows indicate days therapy was given.

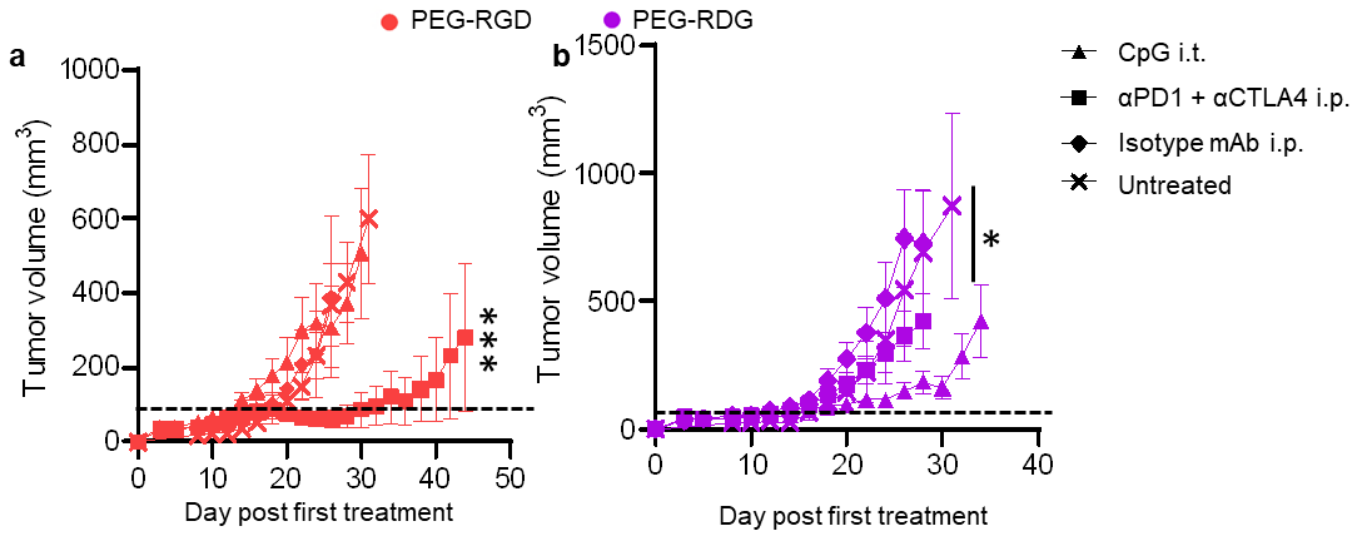


Figure S7: Tumor growth after therapeutic intervention compared to untreated mice. Growth of tumors formed after implantation of 50,000 E0771 cells into C57Bl6 mice within PEG-RGD **(a)** and PEG-RDG **(b)** hydrogels matrix vehicles beginning at tumor implantation and treated with i.t. CpG, i.p. anti-PD1 + anti-CTLA4, i.p. isotype mAb control, or untreated. Treatment groups are from experiment independent of Figure 6; untreated group is from gel degradation study. Data represents mean ± s.e.m. with n=5-7 animals implanted with one tumor. Each animal is treated on the day the tumor reaches 100 mm^3 in volume (noted as a dashed line). * indicates significance by repeated measures ANOVA with Tukey's post-hoc test, against all other groups if not specified. * indicates $p<0.05$, *** indicates $p<0.005$.