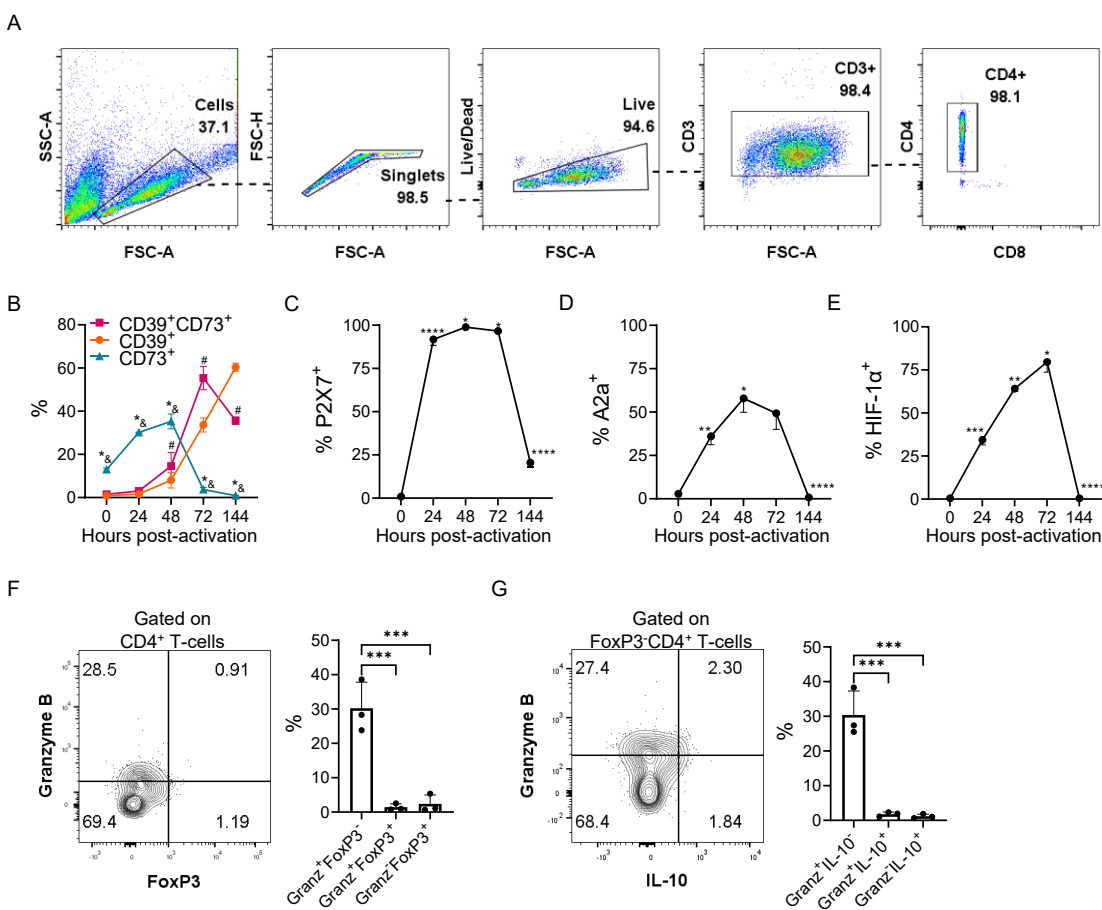
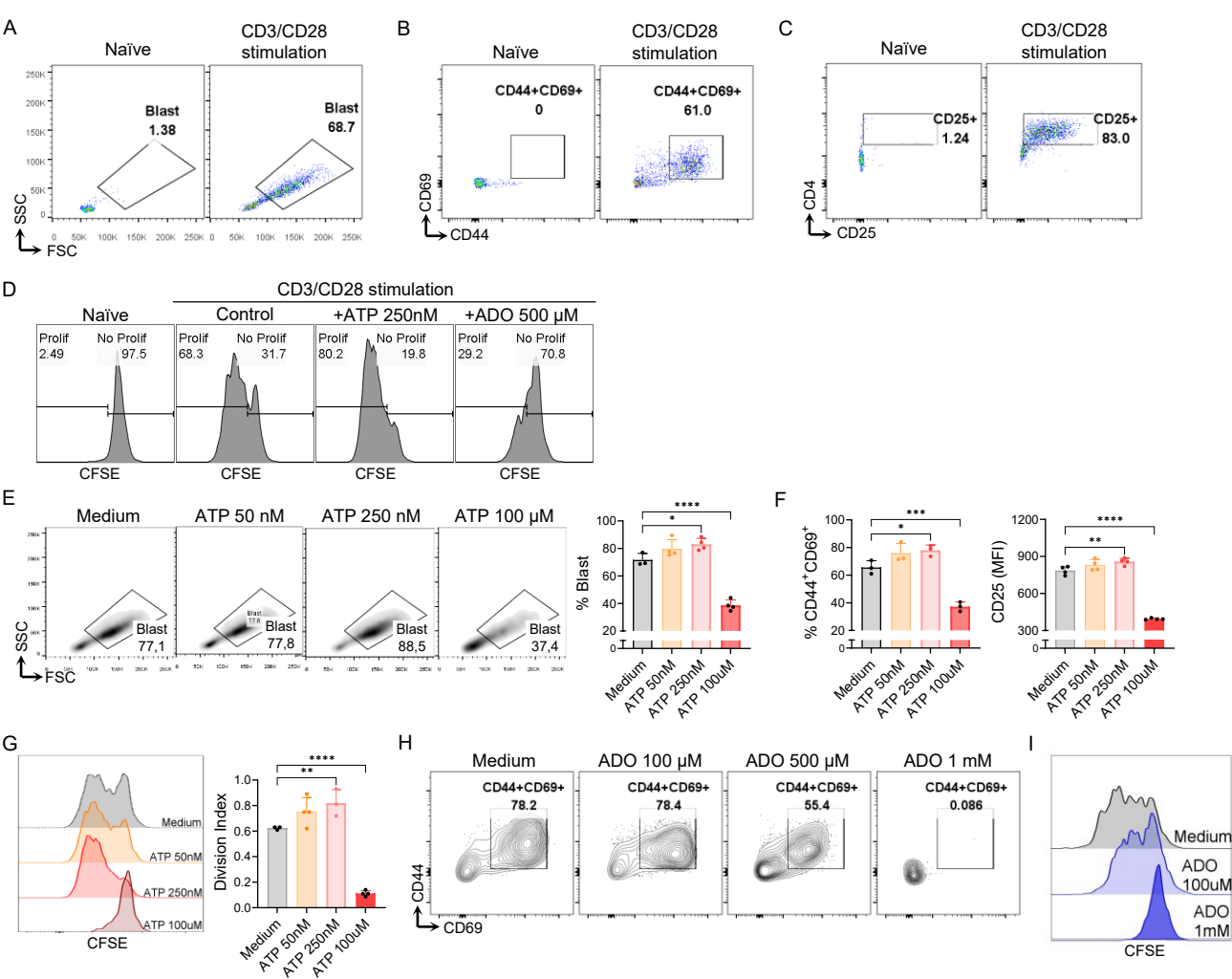
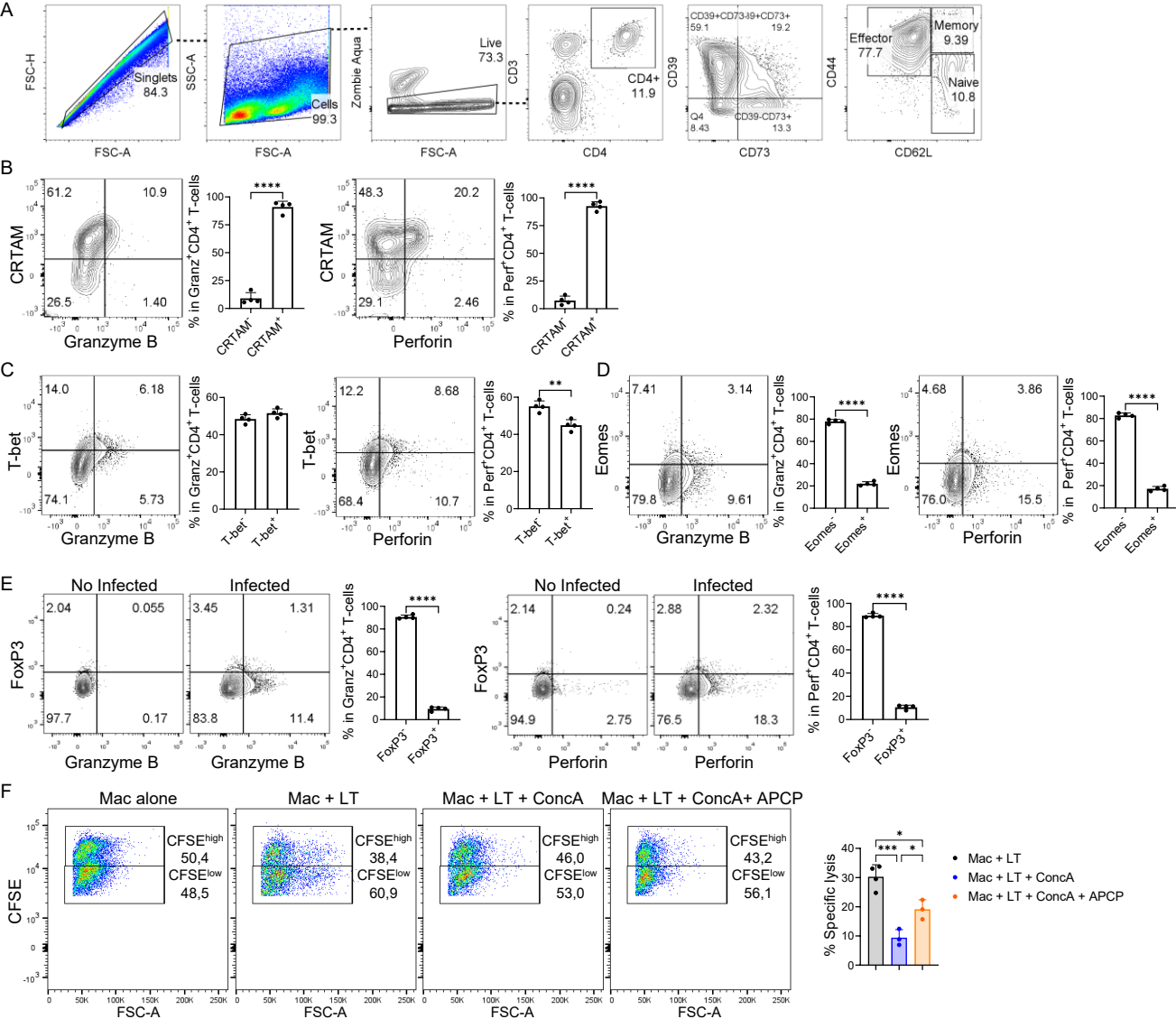




Supplemental Figure 1. In vitro activation of isolated CD4⁺ T-cell. (A) Gating strategy employed to analyze splenic CD4⁺ T-cells by flow cytometry. **(B-E)** Frequency of CD39 and CD73 (B), P2X7R (C), A2aR (D), and HIF-1 α (E) expression in CD4⁺ T-cells at indicated time point post-activation (n = 3/time). **(F-G)** Representative contour plots and frequencies of granzyme B and FoxP3 (F) or IL-10 (G) expression in total CD4⁺ T-cells or FoxP3⁺CD4⁺ T-cells at 72 hours post-stimulation (n = 3/group). Two-ways ANOVA followed by Tukey's post hoc test was conducted for panel B (*CD39⁺ vs CD73⁺, #CD39⁺ vs CD39⁺CD73⁺, &CD73⁺ vs CD39⁺CD73⁺; p > 0.05). Data in all panels were obtained from male mice. One-way ANOVA followed by Tukey's post hoc test was conducted for panels C-G. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.



Supplemental Figure 2. Concentration-dependent maturation effects of extracellular ATP on naïve T-cells. (A-D) Dot plots showing the frequency of blasts (A), the expression of CD44 and CD69 (B), CD25 (C), and representative histograms of the proliferation rate (D) of naïve and 72h-activated CD4⁺ T-cells. **(E-G)** Frequencies of blasts (E), CD44⁺CD69⁺, CD25 expression (F), and proliferation rate (G) in CD4⁺ T-cells stimulated in the presence of ATP (50 nM, 250 nM, or 100 μ M) or maintained in medium alone for 72 h ($n = 4$ /group). **(H-I)** Representative CD44 and CD69 expression (H) and histograms of the proliferation (I) in CD4⁺ T-cells stimulated in the presence of ADO (100 μ M, 500 μ M, or 1 mM) or maintained in medium alone for 72 h. Data in all panels were obtained from male mice. One-way ANOVA followed by Tukey's post hoc test was conducted for panels E-G. Only the statistically significant differences versus medium are shown. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.



Supplemental table 1. List and sequences of RT-PCR primers used.

Primer	Forward Sequence	Reverse Sequence
<i>Hprt</i>	CCCAGCGTCGTGATTAGC	GGAATAAACACTTTTTCCAAATCC
<i>Ldha</i>	TGGCAGACTTGGCTGACAG	ACCTTCACAACATCCGAGATTC
<i>Vegfa</i>	TAGAGTACATCTTCAAGCCG	TCTTTCTTTGGTCTGCATTC
<i>P2rx7</i>	AATCGGTGTGTTTCCTTTGG	CCGGGTGACTTTGTTTGTCT
<i>Entpd1</i>	AGCTGCCCCTTATGGAAGAT	TCAGTCCCACAGCAATCAAA
<i>Cd73</i>	AGAGTTCCTGCAAGTGGGTGGAATC	GACCACTCTGTTCCAGGGCTTTCG
<i>A2AR</i>	CCATCCGAATTCCACTCCGGT	TGACAGCACCCAGCAAATCGC
<i>A2BR</i>	GTGCTGGTGCTCACACAGAGC	TTTATACCTGAGCGGGACGCG

Supplemental table 2. List of reagents used.

Reagents	Concentration used	Company	Catalog Number
Adenosine (ADO)	500 μ M	ThermoFisher	A10781
		Sigma-Aldrich	A4036
Adenosine 5'-triphosphate (ATP)	250 nM / 100 μ M	ThermoFisher	J61125
		Sigma-Aldrich	A6419
Adenosine 5'-(α,β -methylene) diphosphate (APCP)	100 μ M	Sigma-Aldrich	M3763
Deferoxamine mesylate salt (DFO)	5 μ M	Sigma-Aldrich	D9533
ZM-241385	1 μ M	Sigma-Aldrich	Z0153
A-438079	25 μ M	Sigma-Aldrich	A9736
CFSE	5 μ M / 0,5 μ M/ 2,5 μ M	eBioscience	65-0850
Recombinant Murine IL-2	20 ng/mL	PeproTech	212-12-50UG
Rat IgG1 kappa Isotype Control	40 μ g/mL	eBioscience	14-4301-82
Mouse IL-6 Recombinant Protein	10 pg/mL	PeproTech	216-16
IL-6 Monoclonal Antibody (MP5-20F3)	5 μ g/mL	eBioscience	16-7061-81
anti-mouse IFN γ (XMG1.2)	40 μ g/mL	BioXCell	BE0055
Concanamycin A	100 nM	Sigma-Aldrich	C9705

Supplemental table 3. List of antibodies used.

Abs to Flow Cytometry	Clone	Company	Catalog Number
Live/Dead		Invitrogen	L34968
		BioLegend	423102
CD45	30-F11	BioLegend	103114
CD3e	17A2	eBioscience	48-0032-82
	145-2C11	BD	553063
CD4	GK1.5	BD	563331
		BioLegend	100410
CD44	OX-49	eBioscience	67-0441-82
	IM7	BioLegend	103007
CD69	FN50	eBioscience	47-0691-82
CD25	PC61	BD	561257
CD62L	MEL-14	BioLegend	104411
CD39	24DMS1	eBioscience	46-0391-82
CD73	eBioTY/11.8 (TY/11.8)	eBioscience	48-0731-82
		BioLegend	127215
P2X7	1F11	BioLegend	148708
A2a	Polyclonal	Invitrogen	PA1-042
HIF-1 α	EP1215Y	Abcam	ab51608
	H1alpha67	Novus Biologicals	NB100-105
Ki67	16A8	BioLegend	652422
Granzyme B	GB11	BioLegend	515403
	QA18A28	BioLegend	396410
Perforin	S16009A	BioLegend	154305
IFN- γ	XMG1.2	BioLegend	505836
anti-rabbit IgG	Poly4064	BioLegend	406414
CD11b	M1/70	eBioscience	11011282
Eomes	X4-83	BD	567168
T-bet	4B10	BioLegend	127215
CRTAM (CD355)	11-5/CRTAM	BioLegend	142014
FoxP3	FJK-16s	Invitrogen	45-5773-82
IL-10	JES5-16E3	eBioscience	17-7101