European Journal of Immunology

Supporting Information

for

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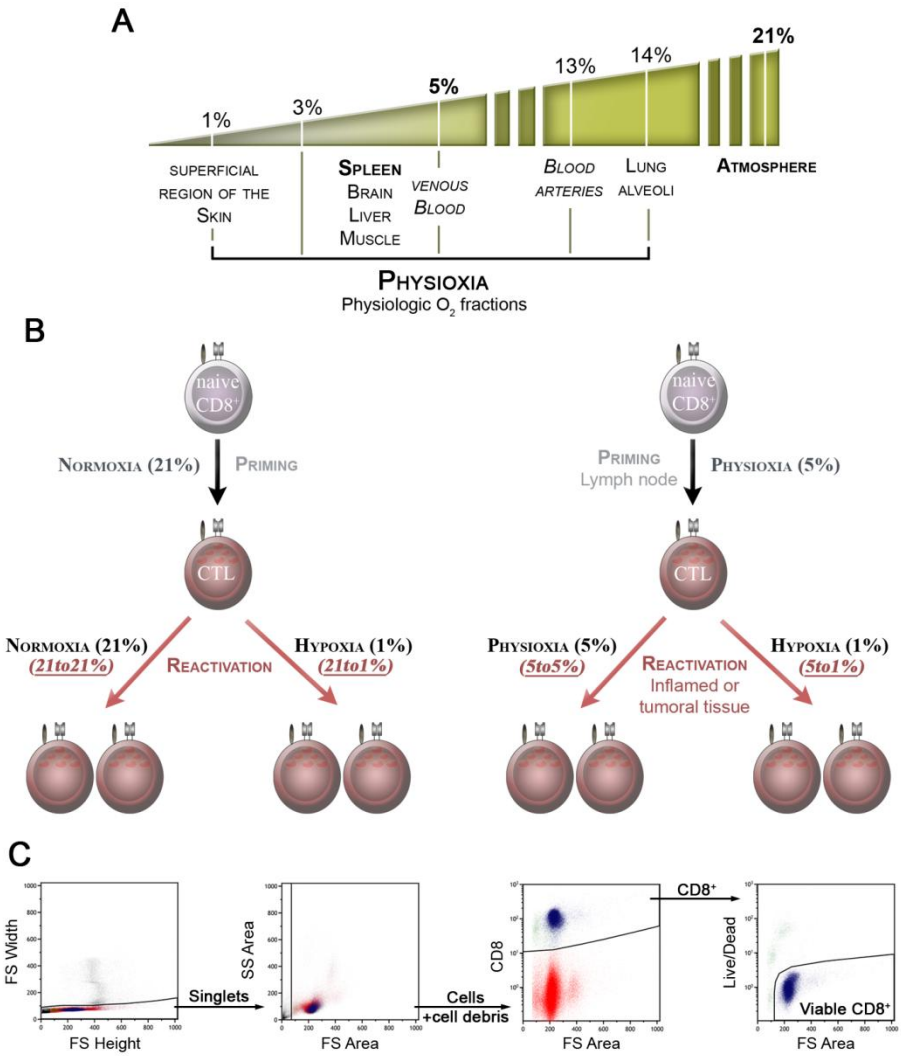
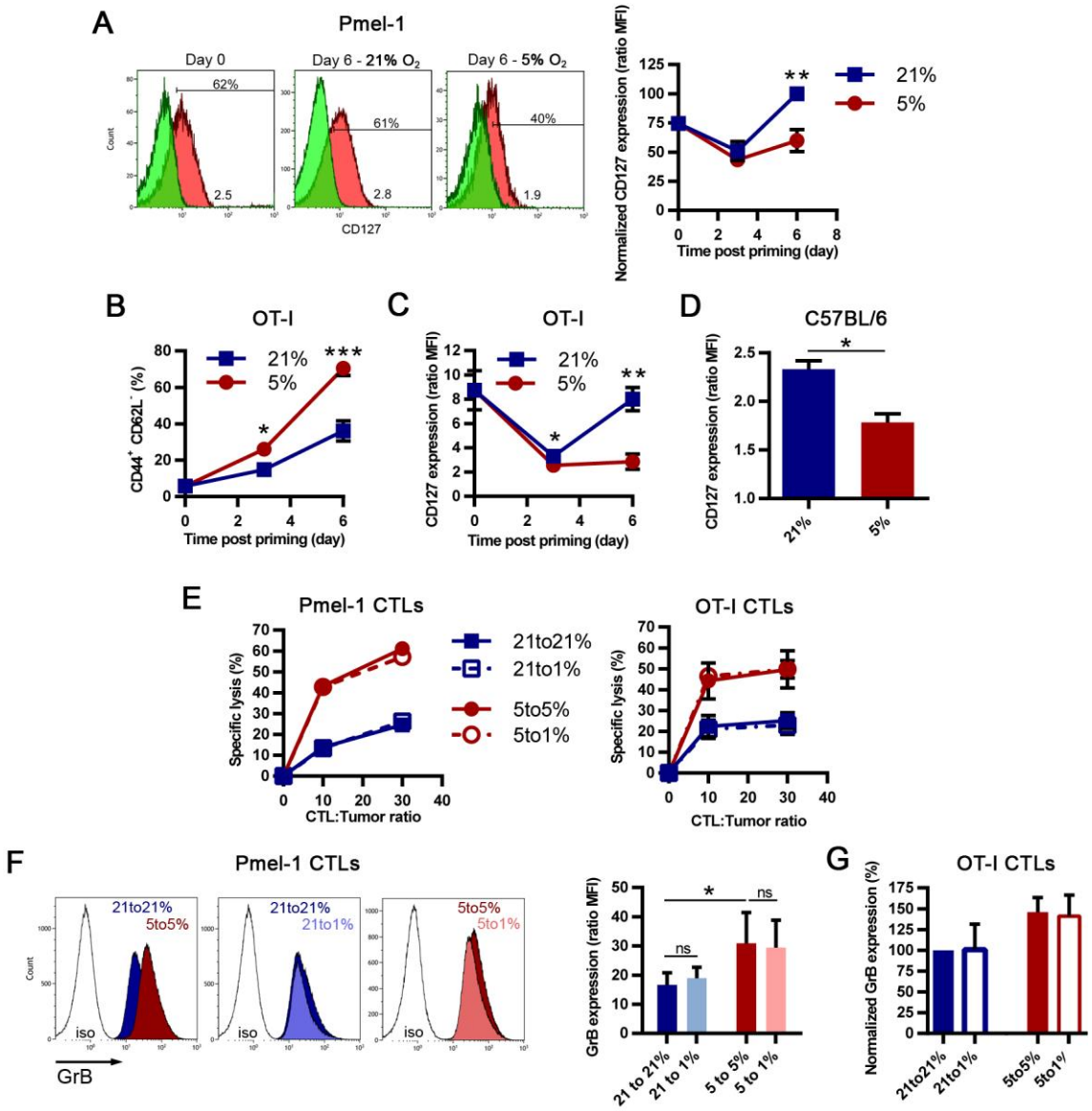
Gene	Target sequence
Hif1a	ACCATGATATGTTTACTAAAGGACAAGTCACCACAGGACAGTACAGGATGCTTGCCAAAAGAGGTGGATATGTCTGGGTTGAAACTCAAGCAACTGTCAT
Epas1 (Hif2a)	ATGTGCTGAGTGAGATCGAGAAGAACGACGTGGTGTTCTCCATGGACCAGACCGAATCCCTGTTCAAGCCACACCTGATGGCCATGAACAGCATCTTTGA
Hif3a	CAGCGACTTCCTCCACTCCACAGACCTATAATCTCTGGATCTTGAATACTTGACTCCCACCTTTACTGTTTGTAGACTGTGCAACTTCTAGCCTTCCCTG
Arnt (Hif1b)	ATTTTGTCATCCTGAAGACCAACAACTTCTAAGAGACAGCTTTCAGCAGGTGGTGAAATTAAAAGGTCAGGTGCTGTCCGTCATGTTCCGATTCCGATCT
Hif1a exonI.1	CCCCGTCCACCCATTTCTACCGCCGGGTGGCTCATTCCCCTCTCCTGTAAGCAAGAAGCCAGAATACATTTTCTCTGCCAGTTTTCTGGGCAAACTGTTA
Slc2a1 (Glut1)	GGACTCCATTTTAGGATTCGCCCATTCCTGTCTCTTCCTACCCAACCACTCAATTAATCTTTCCTTGCCTGAGACCAGTTGGAAGCACTGGAGTGCAGGG
Vegfa	TCTCTCTCTCCCAGATCGGTGACAGTCACTAGCTTGTCCTGAGAAGATATTTAATTTTGCTAACACTCAGCTCTGCCCTCCCTTGTCCCCACCACACATT
Adora2a (A2ar)	GTCCTCACGCAGAGTTCCATCTTCAGCCTCTTGGCTATTGCCATCGACAGATACATCGCCATCCGAATTCCACTCCGGTACAATGGCTTGGTGACGGGTA
Entpd1 (CD39)	TGGCTGTGATAGCTTTGATTGCTGTGGGACTGACCCAGAACAAACCTTTGCCAGAAAATGTTAAGTATGGGATTGTGTTGGATGCGGGGTCATCTCACAC
Nt5e (CD73)	AAGCATGACTCTGGTGATCAAGATATCAGCGTGGTTTCTGAATACATCTCAAAAATGAAAGTAGTTTACCCAGCCGTTGAAGGGCGGATCAAGTTCTCTG
Il2	GCAACTGTGGTGGACTTTCTGAGGAGATGGATAGCCTTCTGTCAAAGCATCATCTCAACAAGCCCTCAATAACTATGTACCTCCTGCTTACAACACATAA
Il2ra (CD25)	AAGGAATTGGTCTATATGCGTTGCTTAGGAAACTCCTGGAGCAGCAACTGCCAGTGCACCAGCAACTCCCATGACAAATCGAGAAAGCAAGTTACAGCTC
Il2rg	ATCCAATGCTCACTGCCTTCCCTTGGGGCTAAGTTTCGATTTCCTGTCCCATGTAACTGCTTTTCTGTTCCATATGCCCTACTTGAGAGTGTCCCTTGCC
Ifng	CTAGCTCTGAGACAATGAACGCTACACACTGCATCTTGGCTTTGCAGCTCTTCCTCATGGCTGTTTCTGGCTGTTACTGCCACGGCACAGTCATTGAAAG
Ifngr1	AAGCATAATGTTACCTAAGTCCTTGCTCTCTGTGGTAAAAAGTGCCACGTTAGAGACAAAACCTGAATCGAAGTATTCACTTGTCACACCGCACCAGCCA
Csf2 (Gmcsf)	AAGTCGTCTCTAACGAGTTCTCCTTCAAGAAGCTAACATGTGTGCAGACCCGCCTGAAGATATTCGAGCAGGGTCTACGGGGCAATTTCACCAAACTCAA
Csf2ra (Gmcsfra)	TCGCGGGAGGCGGGGATACACCGGCGAGTCTCGCCCTTCGGTTGCCGCTGCTGGTTCAGGAGGATGATGGCGCTGCACCACGGTGTCACGCTGGATGTCA
Il10	GGGCCCTTTGCTATGGTGTCCTTTCAATTGCTCTCATCCCTGAGTTCAGAGCTCCTAAGAGAGTTGTGAAGAAACTCATGGGTCTTGGGAAGAGAAACCA
Il10ra	TGTTGTCGCGTTTGCTCCCATTCCTCGTCACGATCTCCAGCCTGAGCCTAGAATTCATTGCATACGGGACAGAACTGCCAAGCCCTTCCTATGTGTGGTT
Il10rb	CTTTACACCTGCGTTTCTCAGCCCCACAAATTGAGAATGAGCCTGAGACGTGGACCTTGAAGAACATTTATGACTCATGGGCTTACAGAGTGCAATACTG
Tgfb1	GGAGTTGTACGGCAGTGGCTGAACCAAGGAGACGGAATACAGGGCTTTCGATTCAGCGCTCACTGCTCTTGTGACAGCAAAGATAACAAACTCCACGTGG
Tgfb2	CCCAAAGCCAGAGTGGCCGAGCAGCGGATTGAACTGTATCAGATCCTTAAATCCAAAGACTTAACATCTCCCACCCAGCGCTACATCGATAGCAAGGTTG
Tgfbr1	TCAGAAGTAGTGGCCAGCTGTGTCTCTAGTAGGACAGTAAAGGCATGAAGCTCAGCCTGTAATCCTGCTACTACAGTAGTACTCCAGAAGTGCCTTGAGG
Tgfbr2	TGTGCAAGTTTTGCGATGTGAGACTGTCCACTTGCGACAACCAGAAGTCCTGCATGAGCAACTGCAGCATCACGGCCATCTGTGAGAAGCCGCATGAAGT
Cd28	TCTGTCTCTCTCTTTCTCTCCTGTGCATATGTCTCCCCTCCCTCACTTCTCTGTCTCTTCCTCTCTCCCTCTCTCTGTCTTTCTCTGTGTGTCTCTCTGT
Tnfrsf9 (CD137)	TCTTCAGAGCAGTTCAAGGGCCTGCTTCTCCTGTTTCCTCTGTGTCAGGCTTTTCAATAAAAAGGCCGTTTAGGAAAGGGACAAAGCACTGTGAGGTGGG
Ctla4	TGGACTCGAGGTCCTGCACCAACTGGCTTGGAAACTAGATGAGGCTGTCACAGGGCTCAGTTGCATAAACCGATGGTGATGGAGTGTAAACTGGGTCTTT
Pdcd1 (PD1)	AGCAGGCTTCCCGGTTTCCTATTGTCACAAGGTGCAGAGCTGGGGCCTAAGCCTATGTCTCCTGAATCCTACTGTTGGGCACTTCTAGGGACTTGAGACA
Bcl2	GGCCTTCTTTGAGTTCGGTGGGGTCATGTGTGTGGAGAGCGTCAACAGGGAGATGTCACCCCTGGTGGACAACATCGCCCTGTGGATGACTGAGTACCTG
Bcl6	ACGTTGTCATCGTGGTGAGCCGTGAGCAGTTTAGAGCCCATAAGACAGTGCTCATGGCCTGCAGCGGCCTGTTCTACAGTATCTTCACTGACCAGTTGAA
Bcl2l1	GAGCAACCGGGAGCTGGTGGTCGACTTTCTCTCCTACAAGCTTTCCCAGAAAGGATACAGCTGGAGTCAGTTTAGTGATGTCGAAGAGAATAGGACTGAG
Gsn (Gelsolin)	GTACCTGTGTCCTGGGACAGTTTCAACAATGGCGACTGCTTCATTCTGGACCTGGGAAACAATATCTATCAGTGGTGTGGCTCTGGCAGCAACAAATTTG
Fas	GGCTCACAGTTAAGAGTTCATACTCAAGGTACTAATAGCATCTCCGAGAGTTTAAAGCTGAGGAGGCGGGTTCGTGAAACTGATAAAAACTGCTCAGAAG
Fasl	CATTTAACAGGGAACCCCCACTCAAGGTCCATCCCTCTGGAATGGGAAGACACATATGGAACCGCTCTGATCTCTGGAGTGAAGTATAAGAAAGGTGGCC
Prdm1 (Blimp1)	CCAATGGCTTGAGCACCATGAACAACATCAATGGTATCAACAACTTCAGCCTCTTCCCTAGGTTGTATCCCGTCTACAGTAACCTCCTTAGTGGCAGCAG
Gata3	CATGCGTGAGGAGTCTCCAAGTGTGCGAAGAGTTCCTCCGACCCCTTCTACTTGCGTTTTTCGCAGGAGCAGTATCATGAAGCCCGAAAGCGACAGATCT
Tbx21 (Tbet)	CCCAGGGCCGCGCGAGGACTACGCATTGCCCGCGGGGTTGGAGGTGTCTGGGAAGCTGAGAGTCGCGCTCAGCAACCACCTGTTGTGGTCCAAGTTCAAC
Foxo1	TTTCCTCAGACTTGGCAACAGCGGCAGCACTTTCCTGTGCAGGATGTTTGCCCAGCGTCCGCAGGTTTTGTGCTCCTGTAGATAAGGACTGTGCCATTGG
Tcf7	CTTTCCCAAGAAGCTCACCAGCATTAACAACTAGTCAATATAGTTGGCCTAAACCCAGTGTGCACCCTTCCTATCAGGCTCTTCCCAGTTCCATTTCCAG
Maf	CTGGCAATGAACAATTCCGACCTGCCCACCAGTCCCCTGGCCATGGAATATGTTAATGACTTCGATCTGATGAAGTTTGAAGTGAAAAAGGAACCGGTGG
Foxp3	CCAGCTCCCGGCAACTTCTCCTGACTCTGCCTTCAGACGAGACTTGGAAGACAGTCACATCTCAGCAGCTCCTCTGCCGTTATCCAGCCTGCCTCTGACA
Lgals3 (Galectin3)	ACAGGAGAGTCATTGTGTGTAACACGAAGCAGGACAATAACTGGGGAAAGGAAGAAAGACAGTCAGCCTTCCCCTTTGAGAGTGGCAAACCATTCAAAAT
Housekeeping Gene	**Target sequence**
Actb	CAGGTCATCACTATTGGCAACGAGCGGTTCCGATGCCCTGAGGCTCTTTTCCAGCCTTCCTTCTTGGGTATGGAATCCTGTGGCATCCATGAAACTACAT
Tubb2a	CGAGTTCGAGGAGGAGGAGGGTGAAGATGAGGCTTGAGAACTTCTCAGATACAGTGTGCACCCTTAGTGAACTTCTGTTGTCCTCCAGCATGGTCTTTCT
Gusb	AATACGTGGTCGGAGAGCTCATCTGGAATTTCGCCGACTTCATGACGAACCAGTCACCGCTGAGAGTAATCGGAAACAAGAAGGGGATCTTCACTCGCCA
Tbp	GTGGCGGGTATCTGCTGGCGGTTTGGCTAGGTTTCTGCGGTCGCGTCATTTTCTCCGCAGTGCCCAGCATCACTATTTCATGGTGTGTGAAGATAACCCA
Eef1a1	AGGACACAGAGACTTCATCAAAAACATGATTACAGGCACATCCCAGGCTGACTGTGCTGTCCTGATTGTTGCTGCTGGTGTTGGTGAATTTGAAGCTGGT

Supporting Information Table 1. List of the different genes and their corresponding target sequences used for RNA analysis by the Nantostring technology.

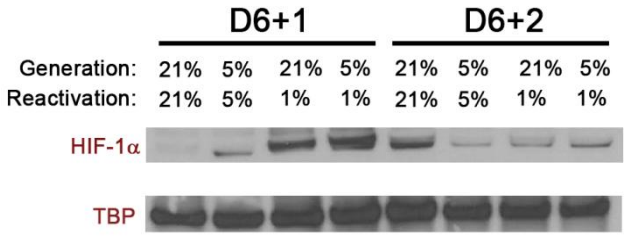


Supporting Information Figure 1. Normoxia, physioxia and hypoxia: experimental approach. (A) Scheme representing oxygen fractions found in different tissues from the body in a physiologic context. (B) Scheme of the *in vitro* experimental approach used all throughout the study. The values of oxygen chosen for normoxia, physioxia and hypoxia were arbitrary selected, based on the oxygen fractions described as in (A) that could be encountered by $CD8^+$ T cells *in vivo*. In this study, naïve $CD8^+$ T cells were divided into two groups. One group was primed under 21% O_2 (normoxia: atmospheric oxygen fraction found in classical incubators that are not found *in vivo*) and the other under 5% O_2 (physioxia: oxygen fraction that can be found in secondary lymphoid organs). Depending on the group, the resulting CTLs were reactivated under two concentrations of oxygen. Those that were primed under 21% O_2 were reactivated under 21% (normoxia; legend: 21to21%) or 1% O_2 (hypoxia; legend: 21to1%), while those that were primed under 5% O_2 were reactivated under 5% (physioxia that could be found in tissues; legend: 5to5%) or 1% O_2 (hypoxia that could be found in inflamed or tumoral tissues; legend: 5to1%). (C) Dot plots representing the gating strategy used to analyze viable $CD8^+$ T cells by flow cytometry.

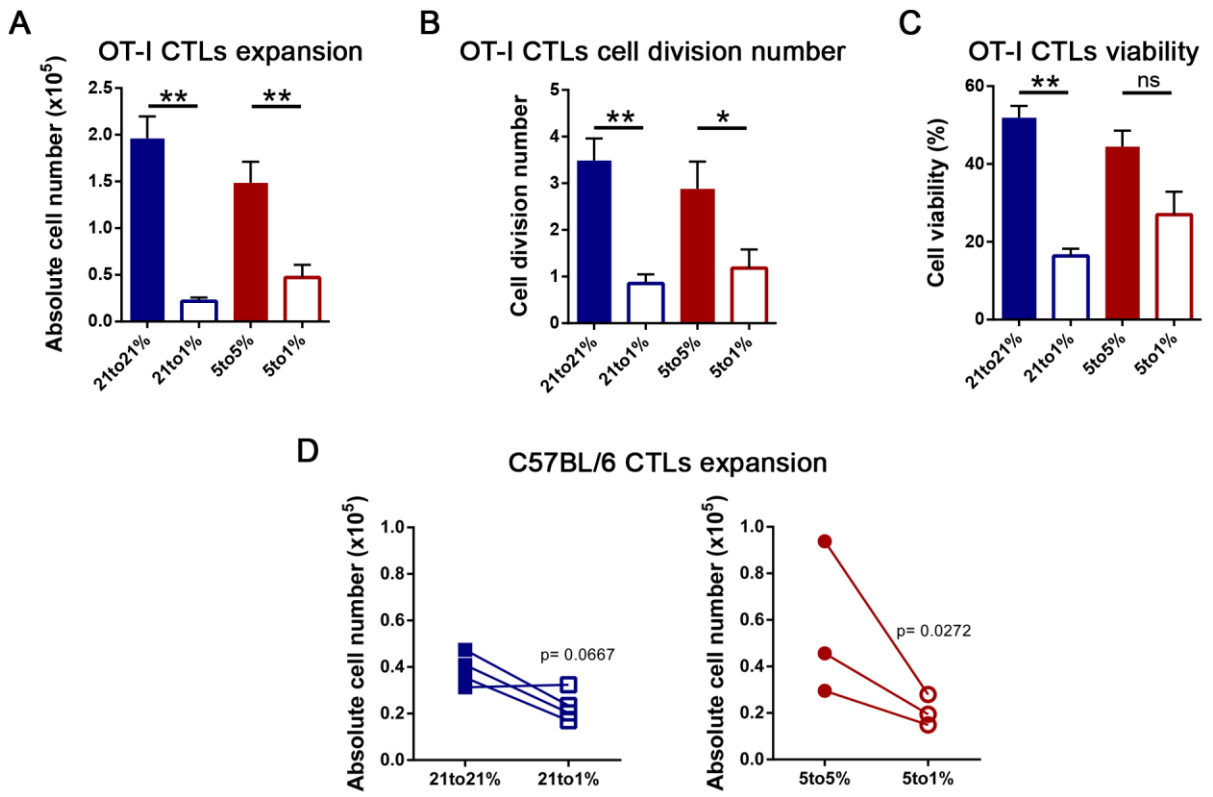


Supporting Information Figure 2. Oxygen tensions during CTL generation impact their phenotype and killing capacities. CTLs were generated under 21% or 5% O_2 from Pmel-1, OT-I or C57BL/6 splenocytes. (A) Histograms show one representative experiment of CD127 expression (green: isotype; red: CD127) from Pmel-1 $CD8^+$ T cells at day 0 or day 6 post priming. Line graph represents the mean ±SEM normalized CD127 expression out of at least 4 independent experiments (n≥4). Results show (B) time-course of the mean $CD44^+$ $CD62L^-$ fraction from OT-I ±SEM from at least three independent experiments (n≥3), (C) time-course of mean CD127 expression from OT-I ±SEM from at least three independent experiments (n≥3) and (D) mean CD127 expression from C57BL/6 CTLs at day 6 post priming +SEM of three independent experiments (n=3). (E) Cytotoxicity, from Pmel-1 and OT-I CTLs generated for 6 days under 21% or 5% O_2, was assessed by coculture

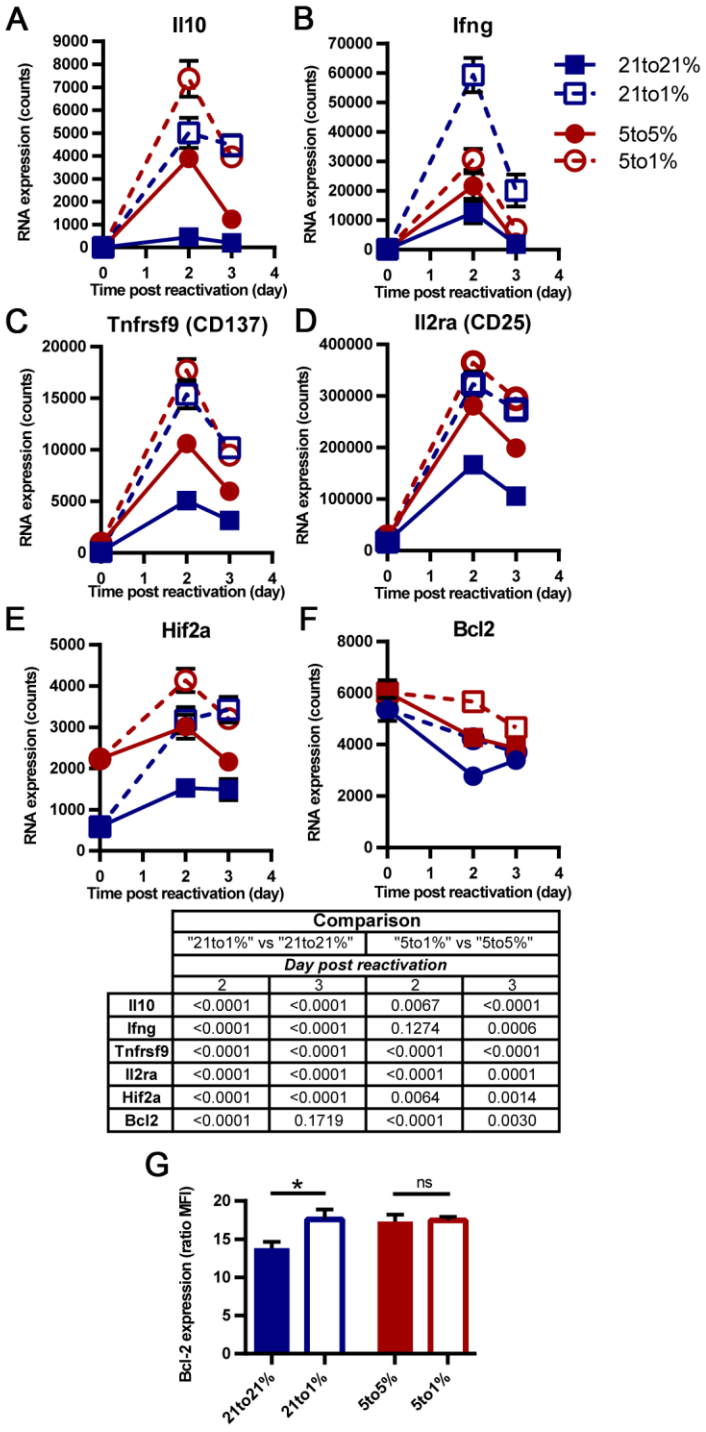
at 10:1 or 30:1 (CTL-to-tumor ratio) under 21%,5% or 1% O_2. Results from Pmel-1 represent the mean specific lysis ±SD of duplicate from a representative experiment out of two independent experiments (n=2), while results from OT-I show the mean specific lysis ±SEM from three independent experiments (n=3). (F-G) CTLs that were preconditioned for 3 days under 21 ("21to21%"), 5 ("5to5%)") or 1% ("21to1%" or "5to1%") were assessed for their GrB content by flow cytometry. (F) Histograms show one representative experiment of a GrB staining from preconditioned Pmel-1 CTLs. Bar graphs show (F) mean GrB expression from preconditioned Pmel-1 CTLs +SEM of at least three independent experiments (n≥4) and (G) mean normalized GrB expression from preconditioned OT-I CTLs +SD of two independent experiments (n=2). ns: not statistically significant, **p*<0.05, ***p*<0.01, ****p*<0.001 (Student's *t*-test).



Supporting Information Figure 3. Low oxygen fraction, and reactivation, promotes HIF-1α stabilization by CTLs. CTLs from Pmel-1 mice generated under 21 or 5% O_2 were reactivated for 1 or 2 days with αCD3/αCD28-coated beads under 21, 5 or 1% O_2. Nuclear fractions of CTLs were obtained using the NE-PER nuclear protein extraction kit (Pierce). Briefly, 15µg of nuclear fraction was loaded and transferred to nitrocellulose membranes (Novex). Anti-HIF-1α (A300-286A; Bethyl) and anti-TBP antibodies (NB500-700; Novus Biologicals) were used.

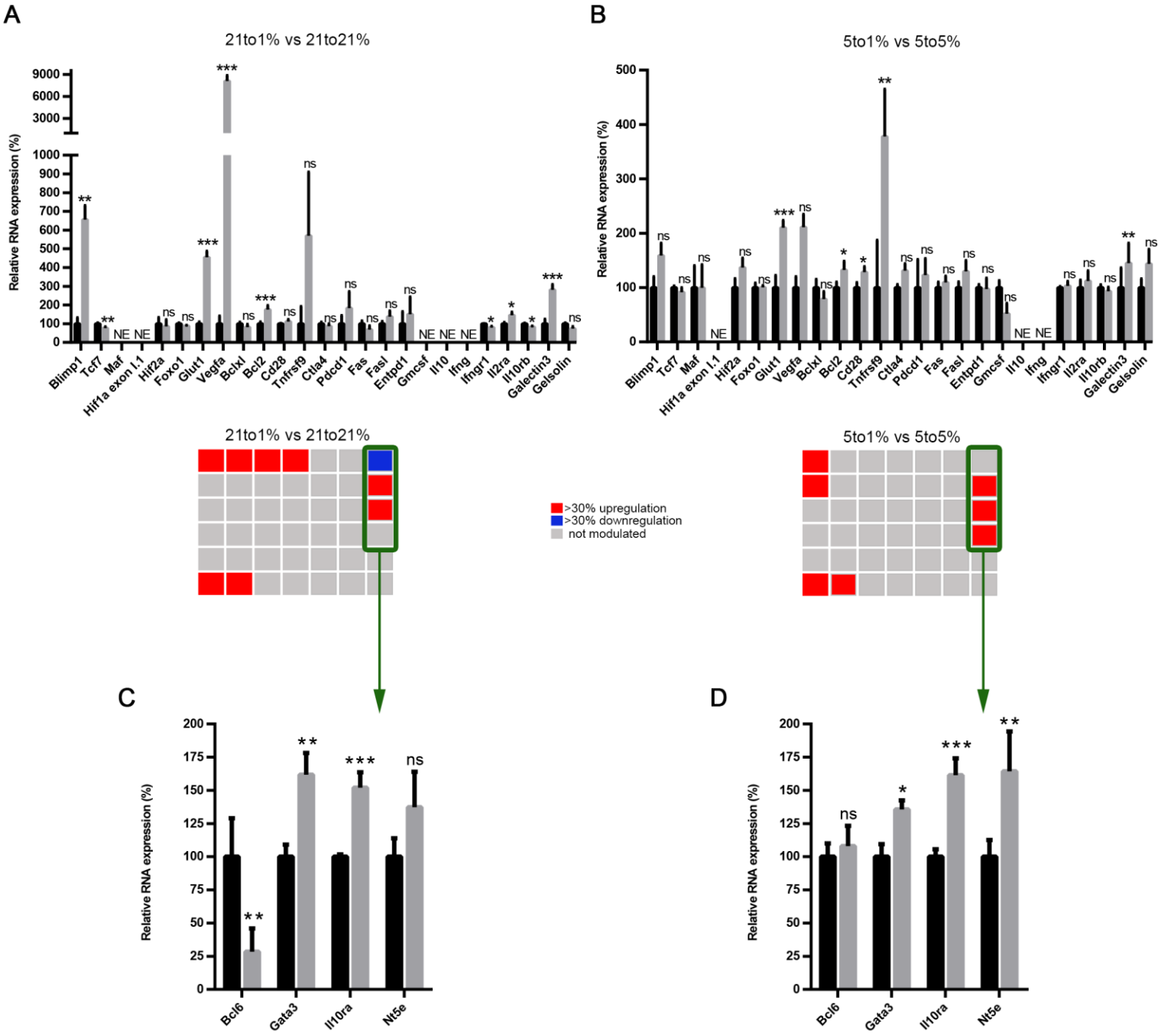


Supporting Information Figure 4. Oxygen tensions impact $CD8^+$ T-cell expansion during reactivation. CTLs generated under 21% (blue) or 5% (red) O_2 from (A-C) OT-I splenocytes or (D) C57BL/6 splenocytes were reactivated for (A-C) three or (D) four days under 21%, 5% or 1% O_2. Results show (A) OT-I mean cell number +SEM, (B) OT-I mean cell division number +SEM, (C) OT-I mean cell viability +SEM, or (D) C57BL/6 absolute cell number from at least three independent experiments (n≥3). ns: not statistically significant, *p<0.05, **p<0.01, ** (Student's t-test).



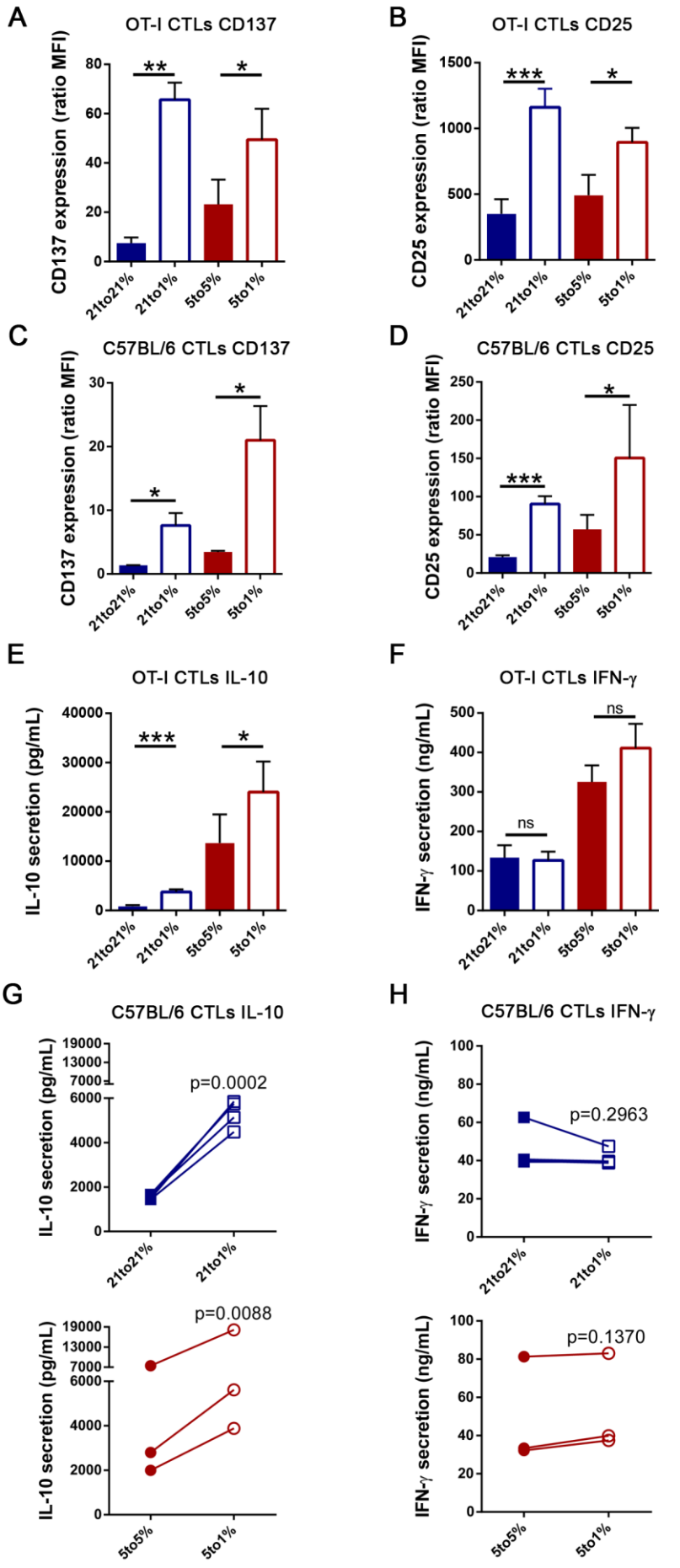
	Comparison			
	"21to1%" vs "21to21%"		"5to1%" vs "5to5%"	
	Day post reactivation			
	2	3	2	3
Il10	<0.0001	<0.0001	0.0067	<0.0001
Ifng	<0.0001	<0.0001	0.1274	0.0006
Tnfrsf9	<0.0001	<0.0001	<0.0001	<0.0001
Il2ra	<0.0001	<0.0001	<0.0001	0.0001
Hif2a	<0.0001	<0.0001	0.0064	0.0014
Bcl2	<0.0001	0.1719	<0.0001	0.0030

Supporting Information Figure 5. Time-course of oxygen-regulated genes in reactivated CTLs. CTLs generated under 21% (squares) or 5% (circles) O_2 from Pmel-1 splenocytes were reactivated for the indicated times under varying oxygen fractions. RNA expression of (A) *il10*, (B) *ifng*, (C) *tnfrsf9 (CD137)*, (D) *il2ra (CD25)*, (E) *hif2a* or (F) *bcl2* from CTLs reactivated under 21% (closed squares with solid line), 5% O_2 (closed circles with solid lines) or 1% O_2 (open squares or open circles with dashed line) was analyzed. Results represent the mean ± SEM of at least four independent experiments (n≥4). Table represents the p-value obtained by three-way ANOVA calculations from (A-F). Comparisons were made between CTL generated at 21% and reactivated at 1% vs CTL generated at 21% and reactivated at 21% O_2 ("21to1%" vs "21to21%") or between CTL generated at 5% and reactivated at 1% vs CTL generated at 5% and reactivated at 5% O_2 ("5to1%" vs "5to5%"). (G) Bcl-2 expression was analyzed by flow cytometry two days post reactivation. Results show the mean Bcl-2 expression +SEM from two independent experiments (n≥3). ns: not statistically significant, *p<0.05. (Student's *t*-test)



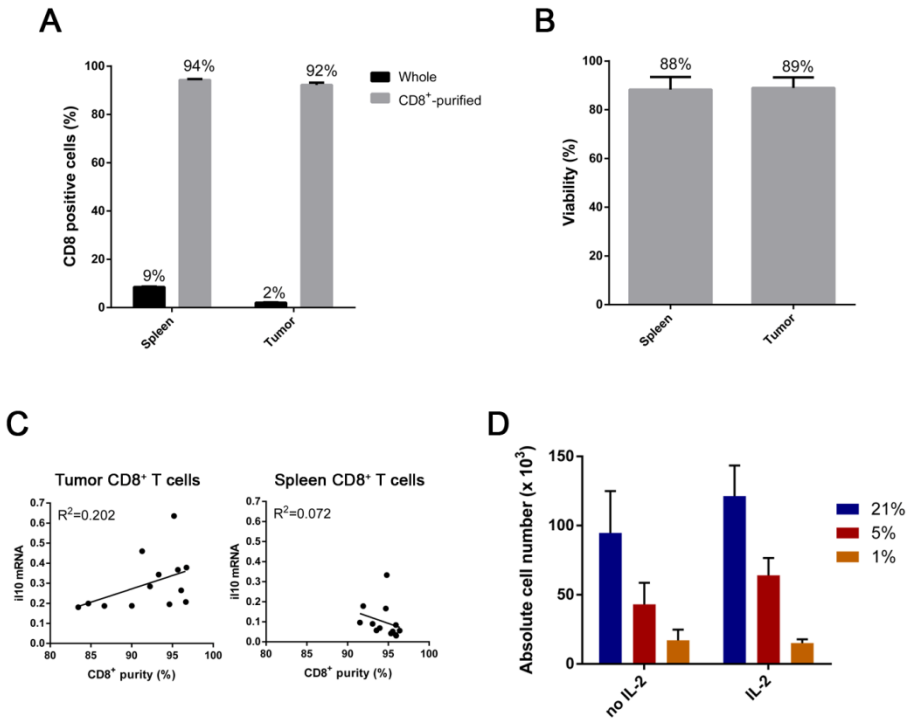
Supporting Information Figure 6. Oxygen tensions impact RNA profile of unstimulated CTLs. CTLs from Pmel-1 mice that were generated under 21 or 5% O_2 were left in culture for 2 days either under 21% ("21to21%"), 5% ("5to5%") or 1% O_2 ("21to1%" and "5to1%"). (A) RNA profile from CTLs left under 21% (black bars) or 1% O_2 (gray bars). (B) RNA profile from CTLs left under 5% (black histograms) or 1% O_2 (gray histograms). Results represent the mean relative gene expression + SEM out of four independent experiments (n=4). The checkerboard represents the 42 genes analyzed (only genes modulated by more than 30% with a $p<0.05$ are colored in red or blue).To display common genes modulated under each condition, genes composing

the checkerboard are organized identically (as in **Fig. 2E-2F**; in an arbitrary fashion). Genes that were not modulated in **Fig. 2E-2F** but that were in this experiment are shown in (C) and (D). Results represent the mean relative gene expression + SEM out of four independent experiments (n=4). NE: Not expressed, ns: not statistically significant, *p<0.05, **p<0.01, ***p<0.001 (Three-way ANOVA).



Supporting Information Figure 7.

Oxygen tensions impact phenotype and IL-10 secretion of CTLs following reactivation. CTLs were generated under 21% or 5% O_2, and were reactivated for (A-C) (E-F) three or (D) (G-H) four days under 21, 5 or 1% O_2. Results show (A) OT-I CD137 mean fluorescence intensity +SEM, (B) OT-I CD25 mean fluorescence intensity +SEM, (C) C57BL/6 CD137 mean fluorescence intensity +SEM, (D) C57BL/6 CD25 mean fluorescence intensity +SEM, (E) OT-I mean IL-10 secretion +SEM, (F) OT-I mean IFN-γ secretion +SEM, (G) C57BL/6 IL-10 secretion and (H) C57BL/6 IFN-γ secretion out of at least three independent experiments (n≥3). ns: not statistically significant, **p*<0.05,**p<0.01, ****p*<0.001. (Student's *t*-test)



Supporting Information Figure 8. IL-2 supplementation does not reverse the hypoxia-induced decrease of $CD8^+$ TILs expansion following reactivation. $CD8^+$ T cells were purified from spleen and tumor of E.G7-OVA-bearing mice. (A) Mean fraction of $CD8^+$ cells among viable cells from spleen and tumor before ("Whole") or after CD8 purification ("$CD8^+$-purified") + SEM out of at least six independent experiments (n=17). (B) Mean cell viability of $CD8^+$ T cells after CD8 purification +SEM out of at least six independent experiments (n=17). (C) Scatter graphs show correlation between $CD8^+$ purity and *il10* mRNA expression (five independent experiments, n=13). R^2 shows correlation coefficient. (D) $CD8^+$ TILs were reactivated for three days in the absence ("no IL-12") or presence ("IL-2") of 100 IU/mL IL-2. Results show mean absolute cell number +SEM from three independent experiments (n=4).