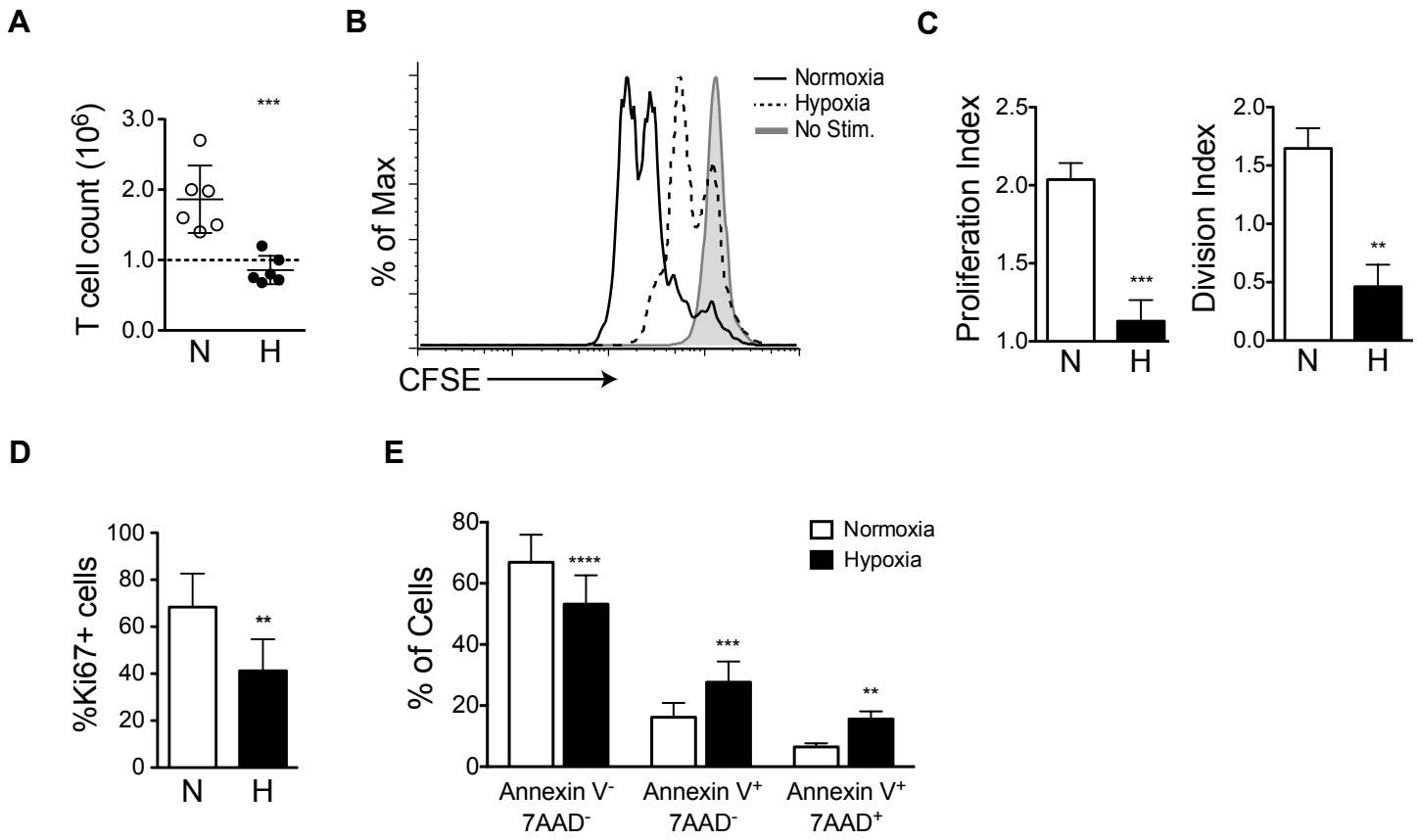
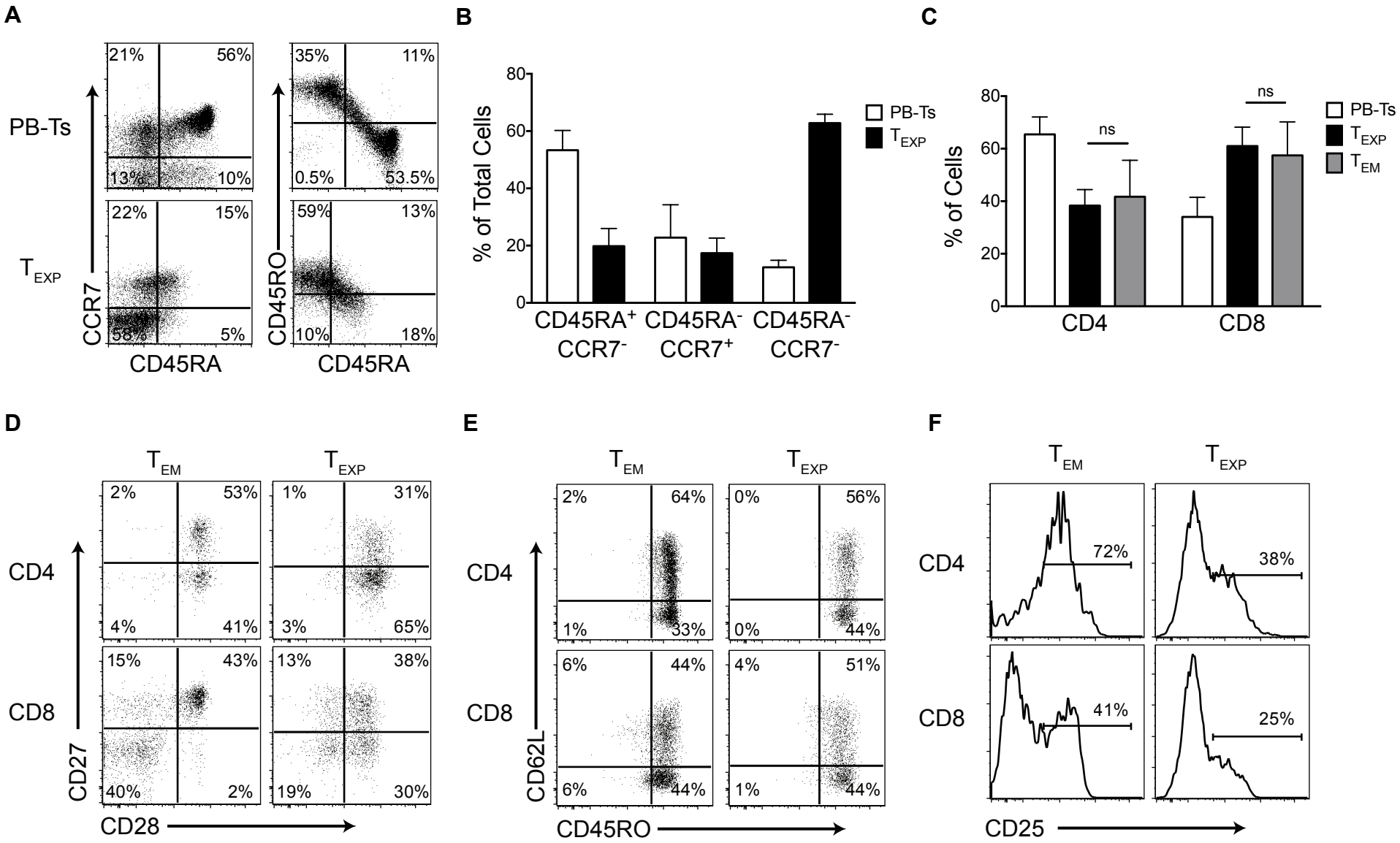


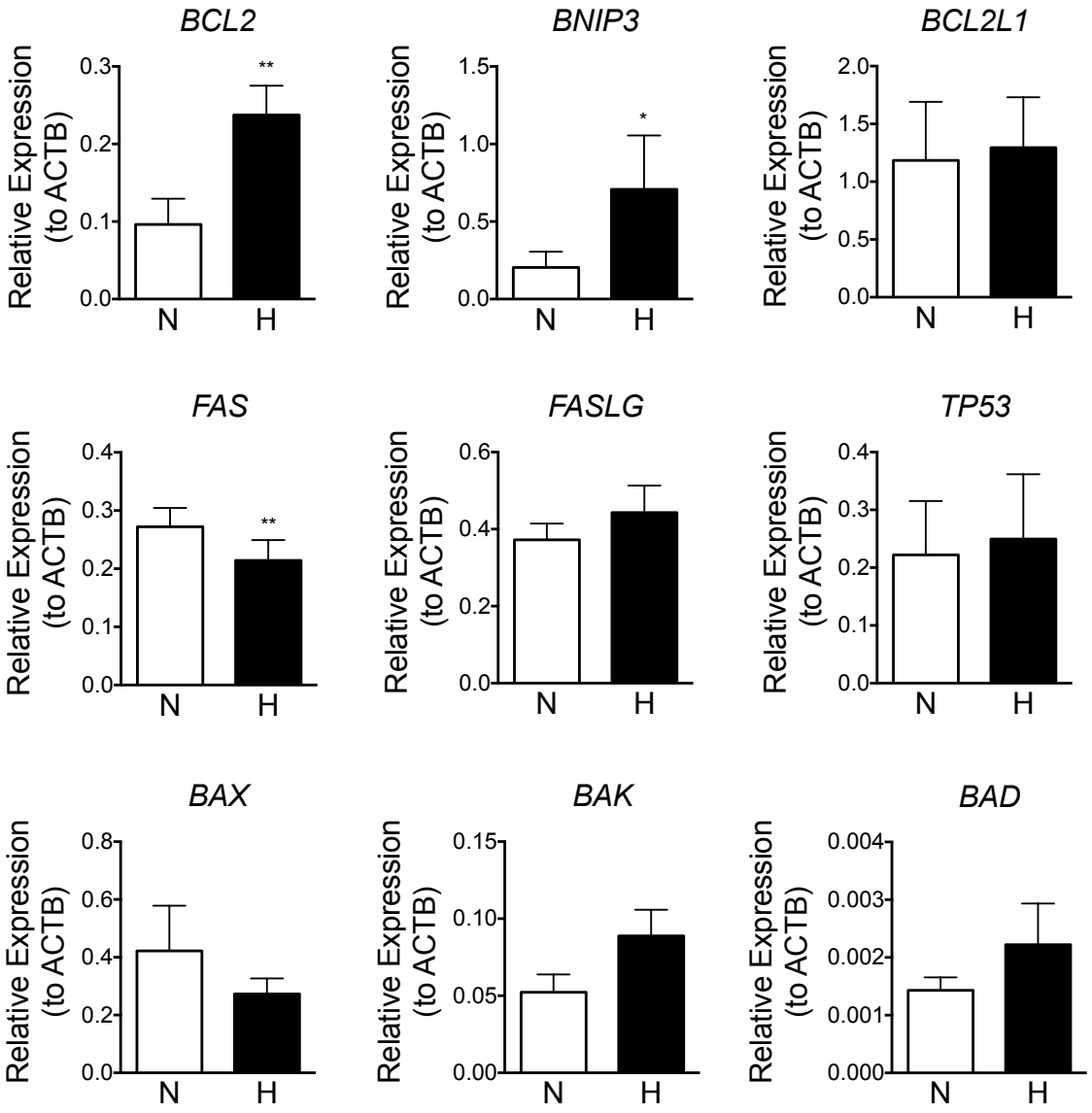
Supplementary Figure 1

Supplementary Figure 1. Hypoxia impairs proliferation and survival of human peripheral blood T cells (PB-Ts). PB-Ts were activated with OKT3/a-CD28 Abs in either normoxia (N) or hypoxia (H). (**A**) Cell counts of PB-Ts at 72 hrs after activation. Dashed line indicates cell counts of plated cells. N=6; ***: p=0.0007 (paired Student's t-test). (**B**) CFSE dilution of CFSE-labeled PB-Ts at 72 hrs. (**C**) Quantitative analysis of the CFSE dilution in (**B**). The proliferation and division indexes were calculated using Flowjo. N=6; ***: p=0.0001; **: p=0.003 (paired Student's t-test). (**D**) Expression of Ki67 in PB-Ts at 72 hrs. N=4; **: p<0.01 (paired Student's t-test). (**E**) Annexin-V and 7-AAD staining of PB-Ts at 72 hrs. N=6; ****: p<0.0001; ***: p=0.0001; **: p=0.0012 (two-way ANOVA with Bonferroni post-hoc analysis). Error bars indicate standard deviations.



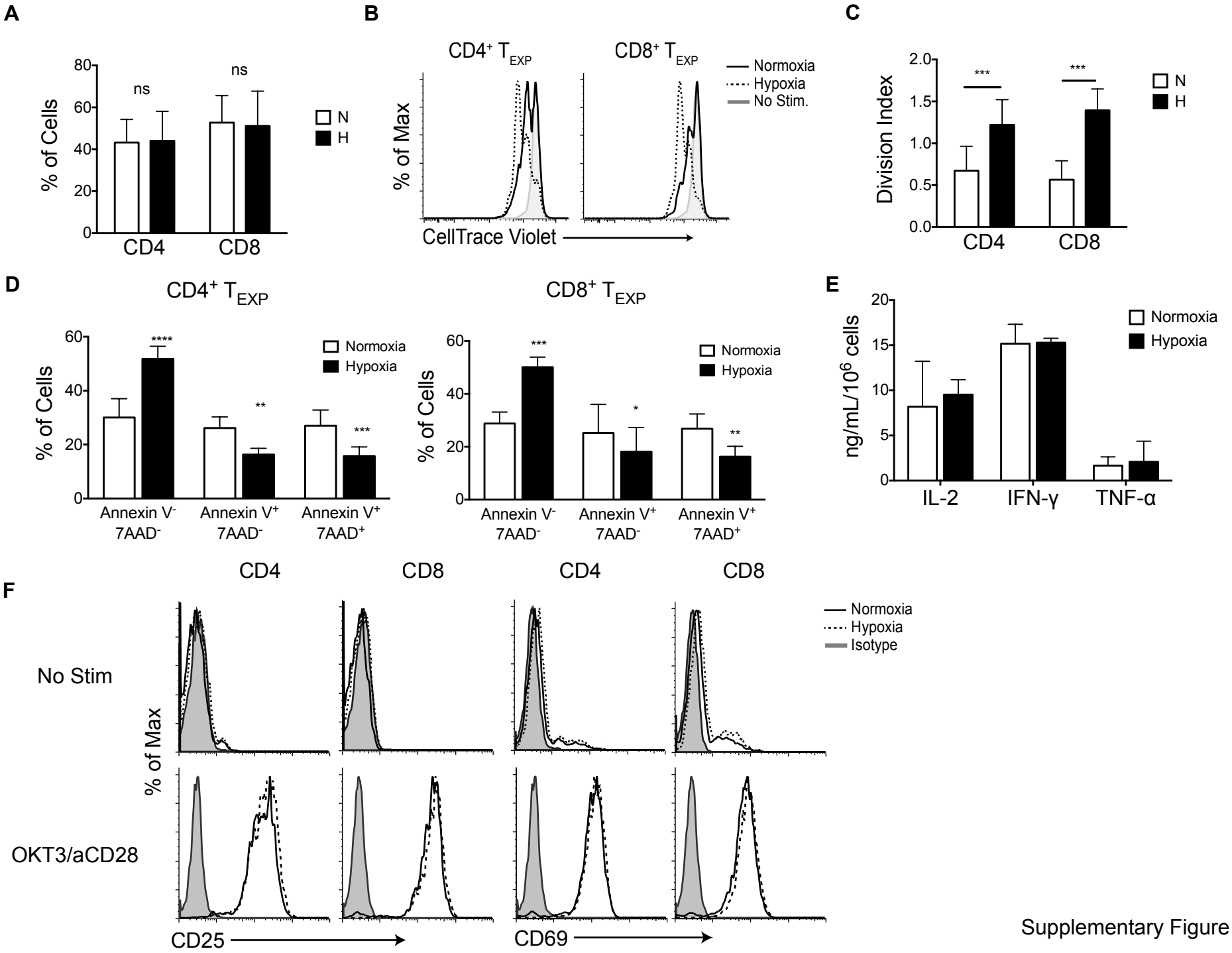
Supplementary Figure 2

Supplementary Figure 2. Phenotypic analysis of PB-Ts and *ex vivo* expanded human effector-memory T cells (T_{EXP}). (**A**) Expression of CD45RA and CCR7 on PB-Ts (top) and T_{EXP} (bottom). (**B**) Percentage of $CD45RA^{+}CCR7^{+}$, $CD45RA^{-}CCR7^{+}$ and $CD45RA^{-}CCR7^{-}$ cells in PB-T and T_{EXP} populations. (**C**) Percentage of $CD4^{+}$ and $CD8^{+}$ cells in PB-T, T_{EXP} and FACS-sorted T_{EM} populations. N=3 (**D-F**) Expression of memory-related surface markers CD27, CD28, CD45RO, CD62L and CD25 on $CD4^{+}$ or $CD8^{+}$ FACS-sorted T_{EM} and T_{EXP} cells. N=3.



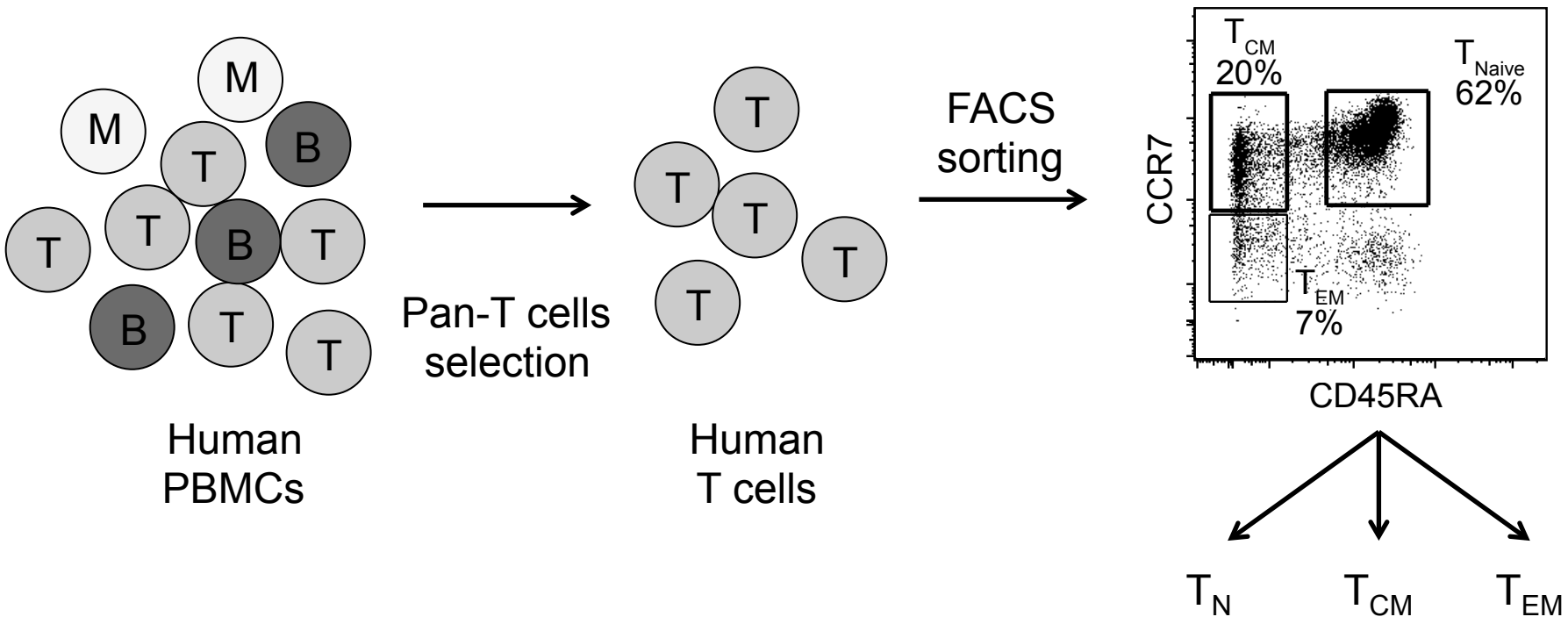
Supplementary Figure 3

Supplementary Figure 3. Apoptosis gene expression profile. Expression of 3 anti-apoptotic (*BCL2*, *BNIP3* and *BCL2L1*) and 6 pro-apoptotic genes (*FAS*, *FASLG*, *TP53*, *BAX*, *BAK* and *BAD*) in T_{EXP} that were activated with OKT3/a-CD28 Abs in either normoxia (N) or hypoxia (H) for 24 hrs. N=4, **: p=0.0076 for *BCL2*; *: p=0.0269 for *BNIP3*; **: p=0.0024 for *FAS (*paired Student's t-test).



Supplementary Figure 4

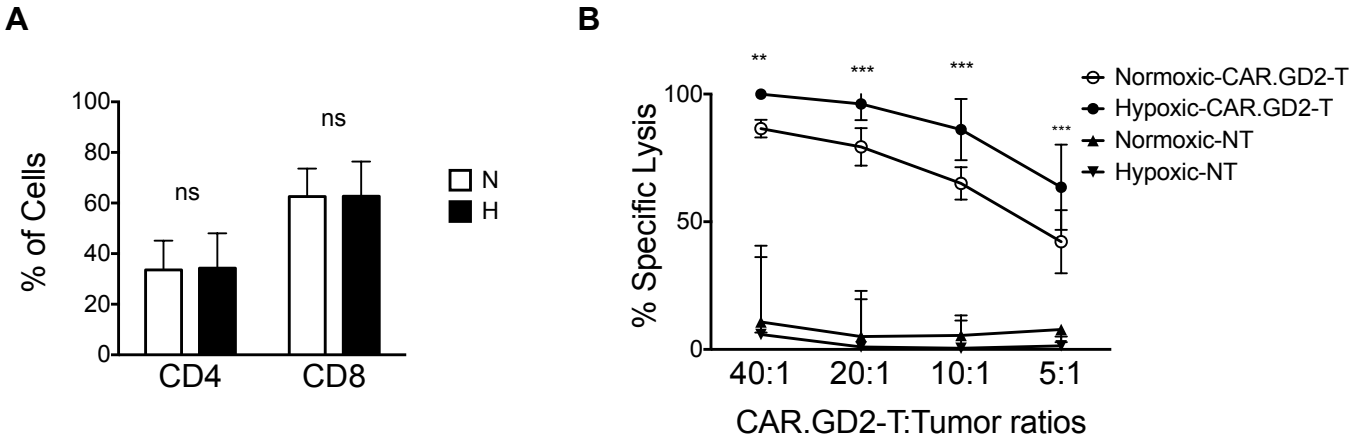
Supplementary Figure 4. Hypoxia does not affect CD4/CD8 ratio, cytokine production and expression of activation markers in T_{EXP}. T_{EXP} were activated with OKT3/a-CD28 Abs in either normoxia (N) or hypoxia (H) for 24 hrs. (**A**) Percentage of $CD4^+$ and $CD8^+$ cells. N=4. (**B**) CellTrace Violet (CTV) dilution of CTV-labeled $CD4^+$ or $CD8^+$ T_{EXP} at 72 hrs after activation. N=3. (**C**) The division indexes were calculated using Flowjo. N=3; ***: $p<0.001$ (two-way ANOVA with Bonferroni post-hoc analysis). (**D**) Annexin-V and 7-AAD staining of $CD4^+$ or $CD8^+$ T_{EXP} at 72 hrs after activation. N=3. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$ (two-way ANOVA with Bonferroni post-hoc analysis). (**E**) Secretion of IL-2, IFN-γ and TNF-α. N=4. (**F**) Expression of CD25 and CD69 in T_{EXP} 24 hrs after activation. N=5.



	Starting		After Sorting		
	#PBMCs	# T cells	T_{Naive}	T_{CM}	T_{EM}
Donor 1	130M	40M	14M	6.8M	3M
Donor 2	200M	50M	10.8M	12M	6M
Donor 3	300M	55M	16M	5.9M	1.7M

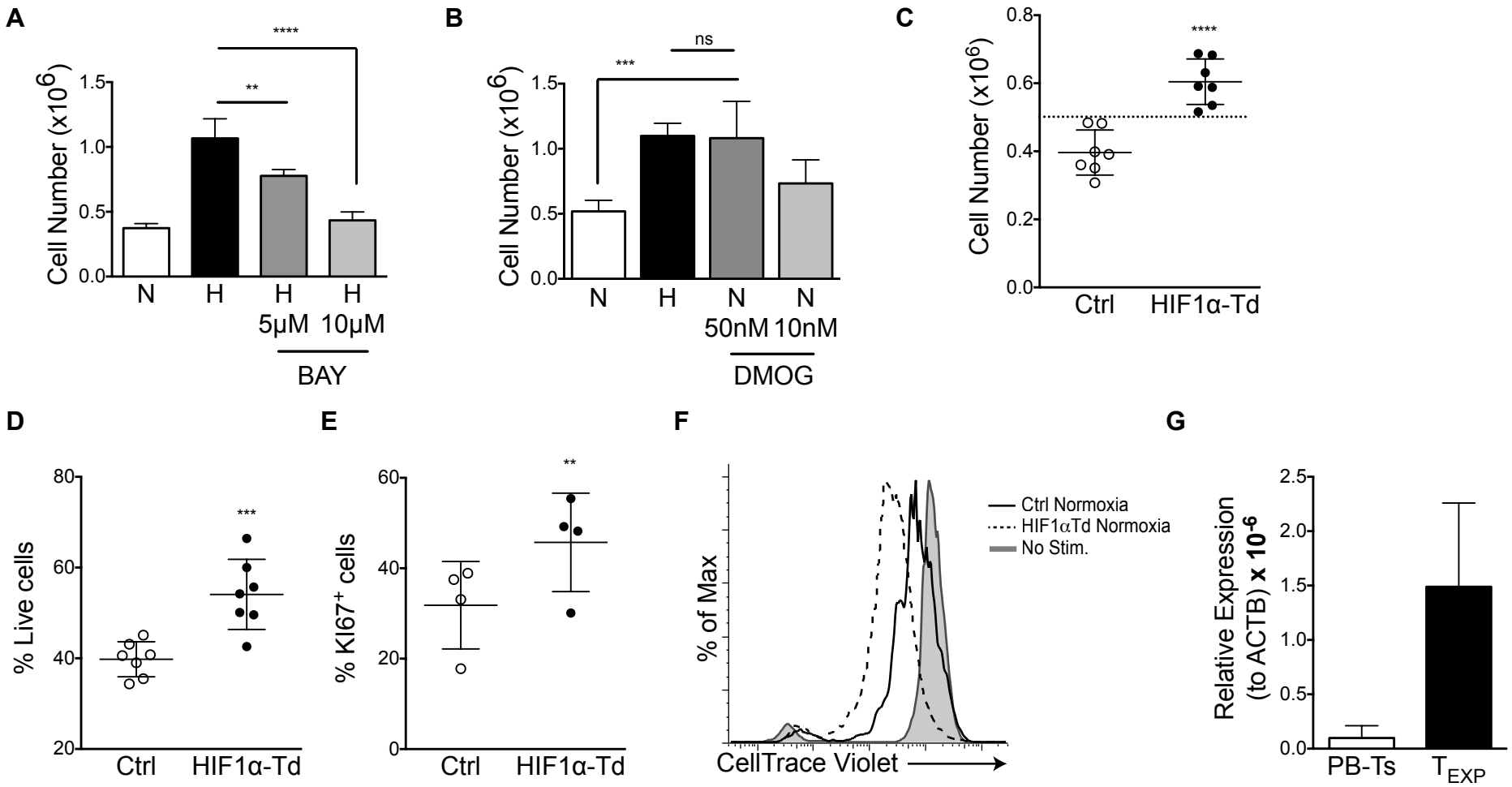
Supplementary Figure 5

Supplementary Figure 5. FACS-sorting of T_N, T_{CM} and T_{EM} from human PBMCs and cell number before and after sorting.



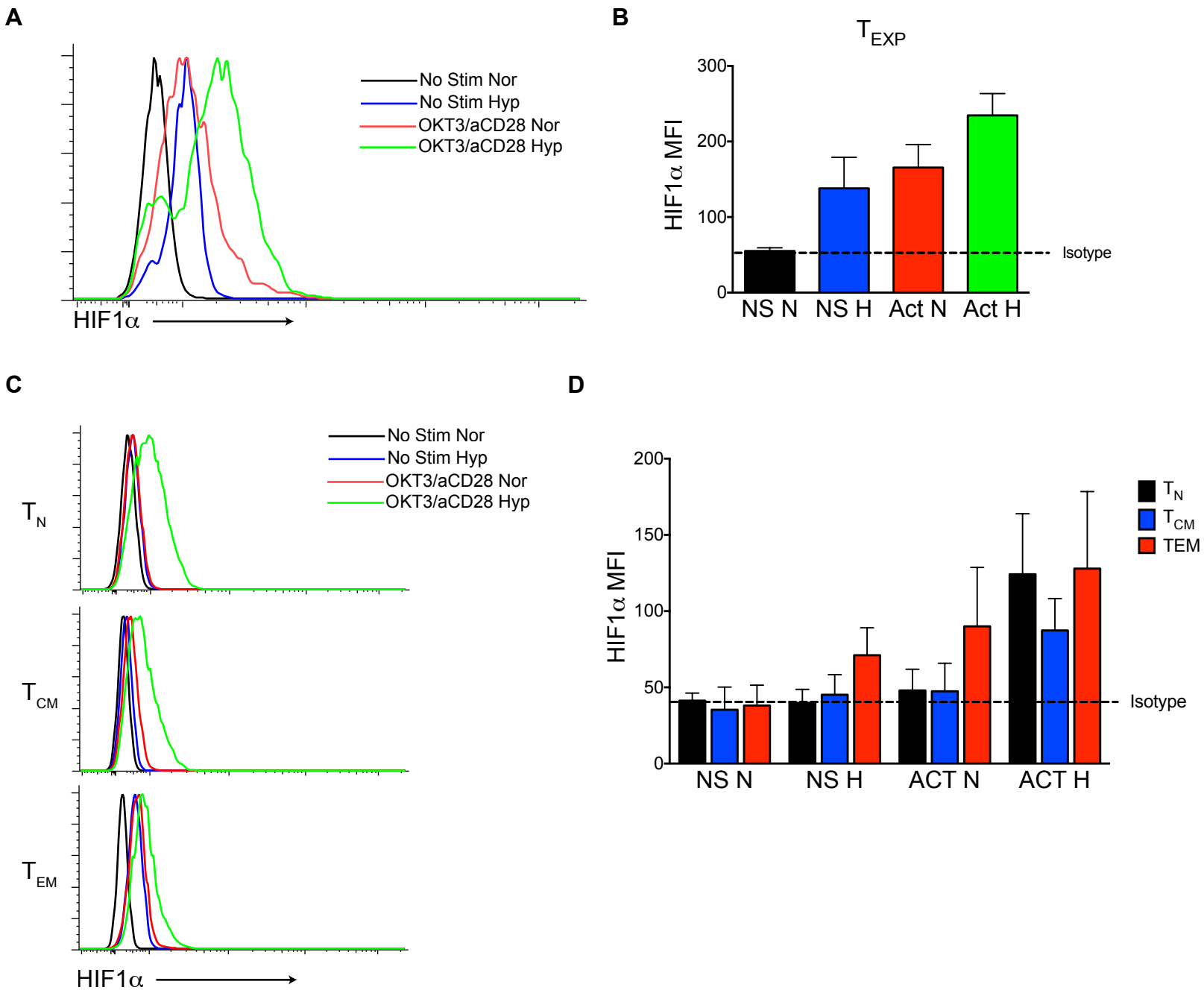
Supplementary Figure 6

Supplementary Figure 6. Hypoxia enhances per-cell cytotoxicity of CAR.GD2-T. (**A**) CD4 and CD8 composition of CAR.GD2-T co-cultured with LA-N-1 target cells in hypoxia or normoxia for 48 hrs. N=3. (**B**) LA-N-1 GFP^+ cells were labeled with ^{51}Cr and co-cultured with CAR.GD2-T or non-transduced T_{EXP} (NT) in normoxia or hypoxia at 40:1, 20:1, 10:1, 5:1 effector:target ratio. The cytotoxic activity of CAR.GD2-T was determined after 6 hrs of co-culture. N=3. **: $p<0.01$; ***: $p<0.001$ (two-way ANOVA with Bonferroni post-hoc analysis).



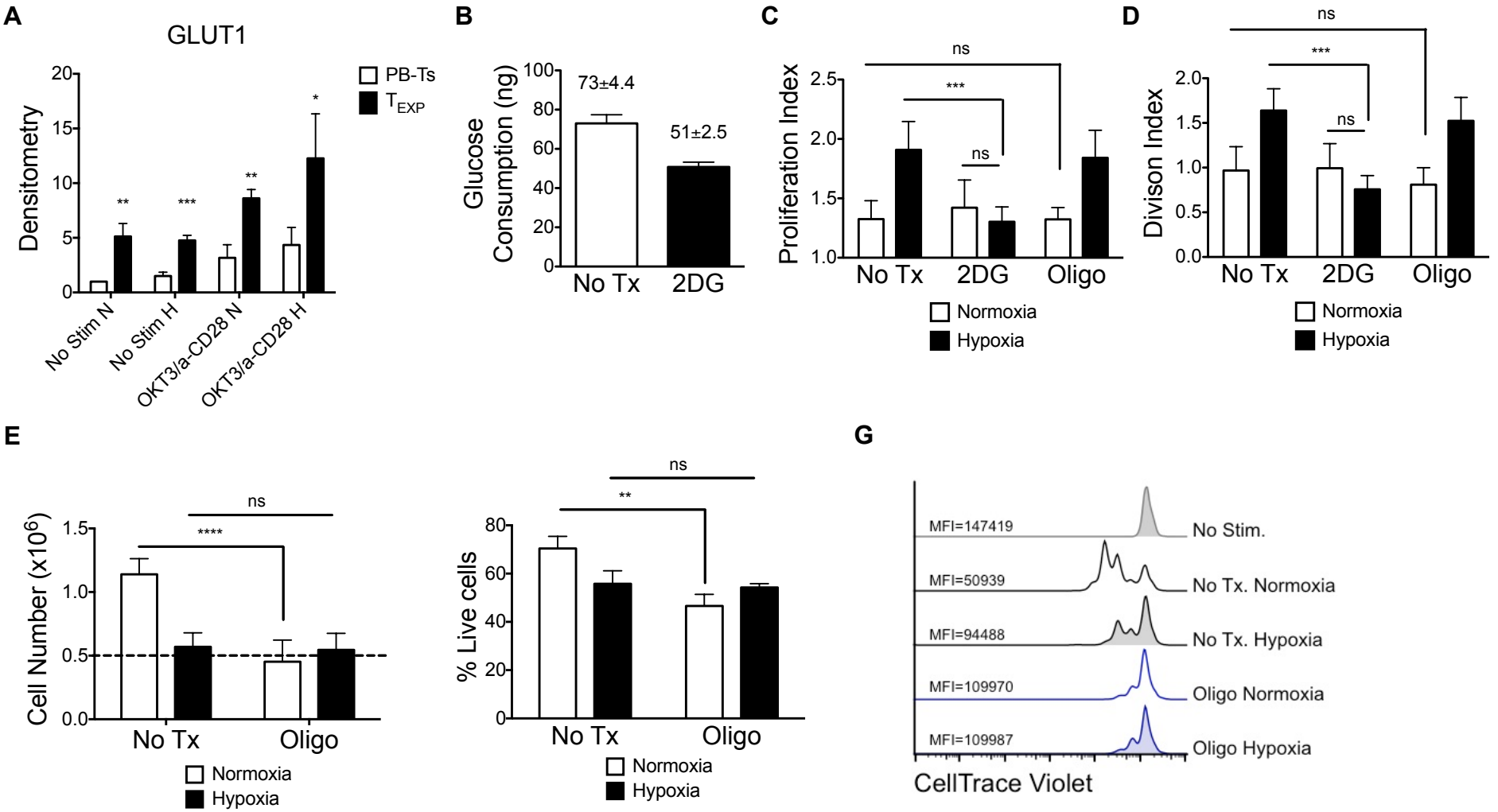
Supplementary Figure 7

Supplementary Figure 7. HIF1α is required and sufficient for enhancing functionality of T_{EXP} in hypoxia. (**A**) Cell counts of T_{EXP} stimulated with OKT3/a-CD28 Abs in normoxia (N) or hypoxia (H) for 72 hrs with or without the HIF1α inhibitor BAY 87-2243 (BAY). N=3; **: p=0.0047; ****: p<0.0001 (one-way ANOVA with Bonferroni post-hoc analysis). (**B**) Cell counts of T_{EXP} stimulated with OKT3/a-CD28 Abs in N or H for 72 hrs with or without the HIF1α activator DMOG. N=3; ***: p=0.0002; ns: p>0.05 (one-way ANOVA with Bonferroni post-hoc analysis). (**C-F**) HIF1α transduced (HIF1α-Td) or mock-transduced (Ctrl) T_{EXP} were stimulated with OKT3/a-CD28 Abs in N. Total cell counts (**C**), cell viability (**D**) and Ki67-expressing cells (**E**) at 72 hrs post stimulation. N=7 for (**C, D**); ****: p<0.0001; ***: p=0.0006; N=4 for (**E**); **: p=0023 (paired Student's t-test). (**F**) CellTrace Violet dilution of labeled HIF1α-Td or Ctrl T_{EXP} at 72 hrs after stimulation. N-3. (**G**) mRNA expression of *EPAS1* (HIF2α) in PB-Ts and T_{EXP}. Expression level is normalized to *ACTB*. Error bars indicate standard deviation.



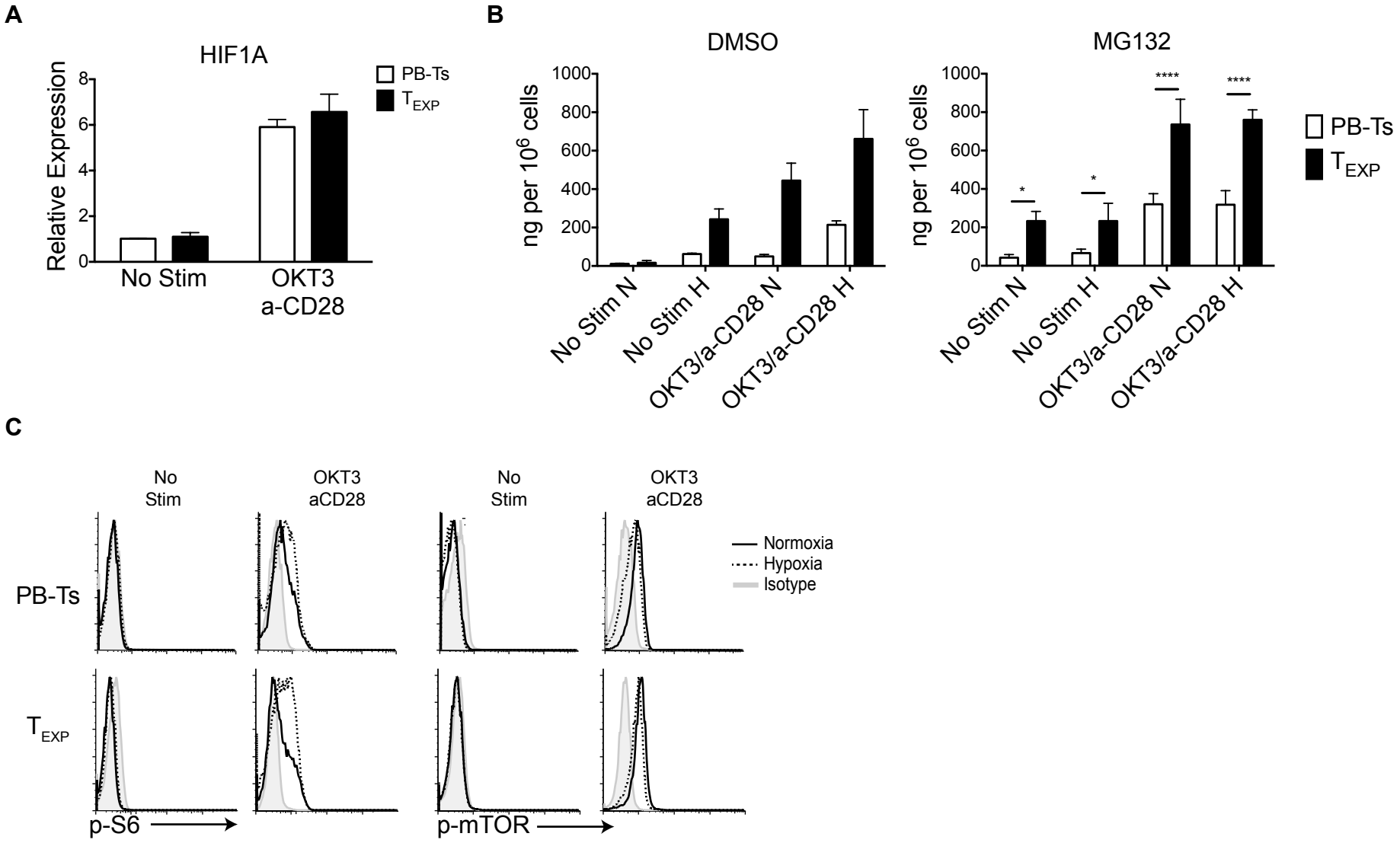
Supplementary Figure 8

Supplementary Figure 8. Intracellular staining of HIF1α in T_{EXP} and T cell memory subsets. T_{EXP} (**A-B**) and FACS-sorted T_N, T_{CM} and T_{EM} (**C-D**) were unstimulated or stimulated with OKT3/a-CD28 Abs in normoxia (N) or hypoxia (H) for 24 hrs and stained for intracellular HIF1α. N=2 for T_{EXP} and N=3 for T memory subsets.



Supplementary Figure 9

Supplementary Figure 9. T_{EXP} display elevated HIF1α expression and glycolytic activity. (**A**) Densitometric analysis of GLUT1 protein in **Fig. 5C**. N=3**. *: p<0.05; **: p<0.01; ***: p<0.005** (two-way ANOVA with Bonferroni post-hoc analysis). (**B**) Glucose consumption of T_{EXP} that were untreated (No Tx) or were treated with 1mM 2DG under conditions of normoxia. N=3. Numbers above the bar indicates mean consumption ± standard deviation. Proliferation index (**C**) and division index (**D**) of T_{EXP} that were untreated or exposed to 1mM 2DG or 50nM oligomycin (Oligo) after stimulation with OKT3/a-CD28 Abs in N or H. (**E,F**) PB-Ts were treated with 50nM oligomycin or left untreated and stimulated with OKT3/a-CD28 Abs in N or H. Cell counts (**E**) and cell viability (**F**) were determined 72 hrs post-activation. N=6. ****: p<0.0001; **: p=0.0011 (two-way ANOVA with Bonferroni post-hoc analysis). (**G**) CellTrace Violet dilution of labeled PB-T at 72 hrs post-stimulation. N=3. Error bars indicate standard deviation.



Supplementary Figure 10

Supplementary Figure 10. Comparison of *HIF1A* mRNA, HIF1α proteasomal degradation, and mTOR activity in PB-Ts and T_{EXP}. (**A**) *HIF1A* mRNA expression in PB-Ts and T_{EXP} that were unstimulated or stimulated with OKT3/a-CD28 Abs in hypoxia (H) for 24 hrs. Expression was normalized against the house keeping control 18S RNA and then standardized to 1.0 in unstimulated PB-Ts. N=3. (**B**) Quantification of HIF1α protein expression by quantitative HIF1α ELISA in PB-Ts and T_{EXP} that were unstimulated or stimulated with OKT3/a-CD28 Abs in the presence of DMSO or 10μM MG132 in normoxia N or H. N=3 for PB-Ts and N=4 for T_{EXP}; *: $p<0.05$; ****: $p<0.0001$ (two-way ANOVA with Bonferroni post-hoc analysis). (**C**) Phosphorylation of S6 and mTOR in PB-Ts and T_{EXP} that were unstimulated or stimulated with OKT3/a-CD28 Abs in N or H for 24 hrs. N=3. Error bars indicate standard deviation.

A

Human HIF1α 3'UTR

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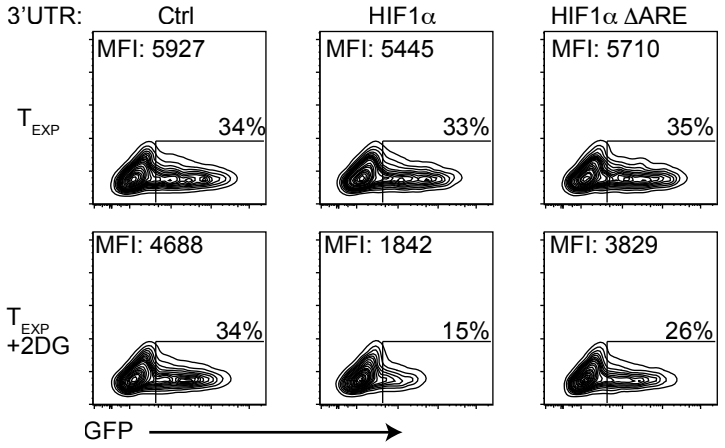
B



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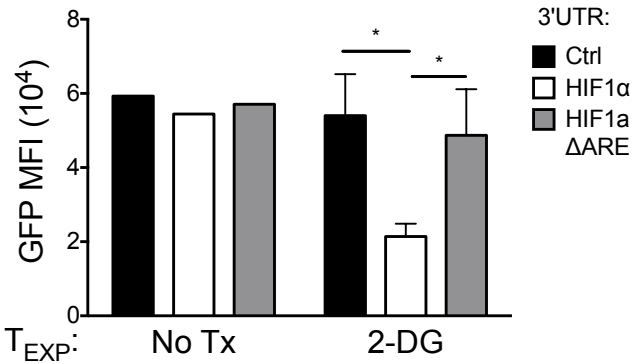
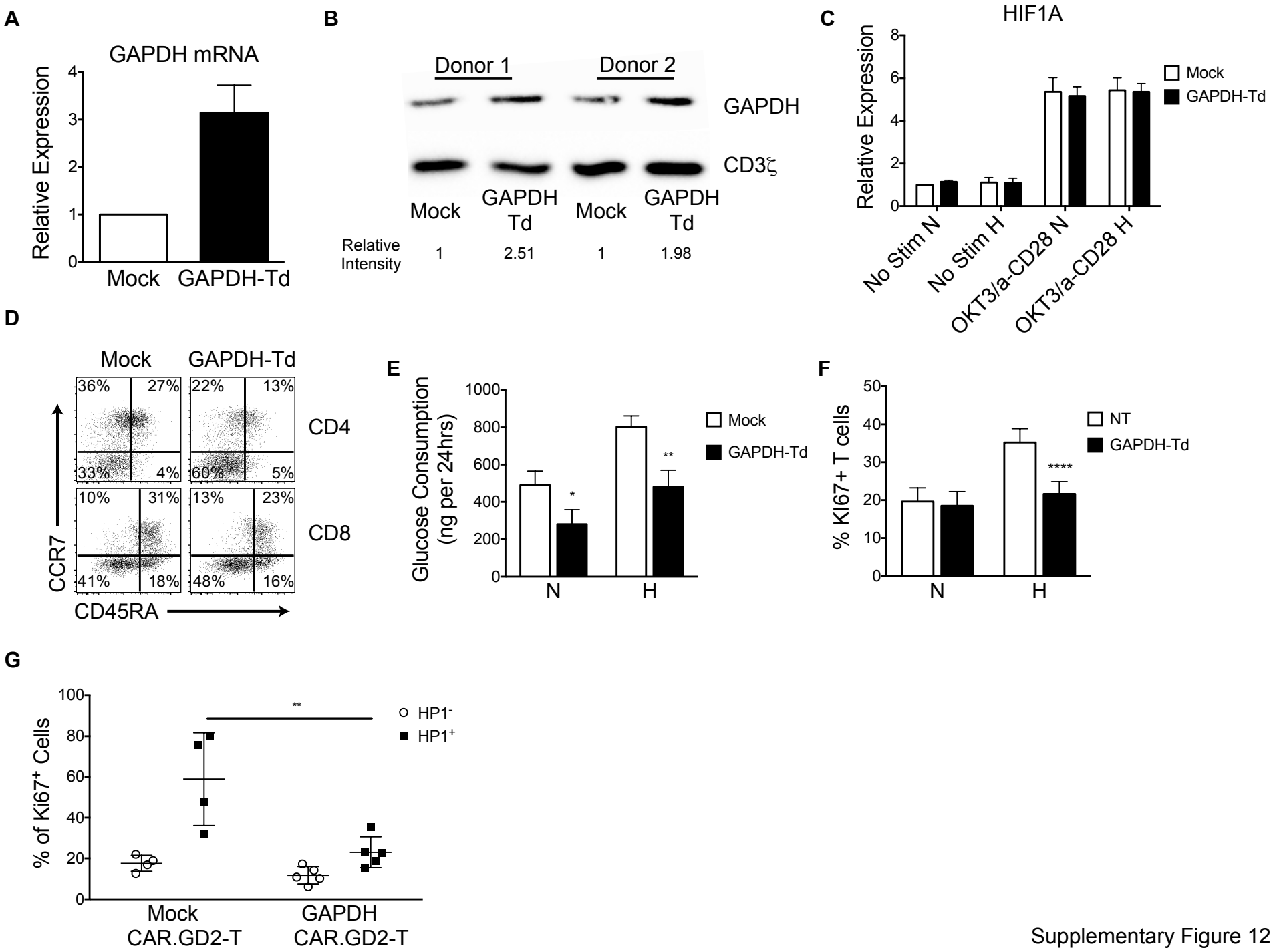


Supplementary Figure 11

Supplementary Figure 11. Regulation of HIF1α expression by 3'UTR (**A**) Sequence of the human *HIF1A* mRNA 3'UTR. Yellow highlights pentamer AREs and red and blue highlight two consecutive nonamer AREs. ARE, AU-rich element. (**B**) T_{EXP} were cultured with IL-2 with or without 1mM 2DG for 48 hrs and subsequently transfected with reporter constructs containing different 3'UTR sequences by nucleofection. Expression of GFP reporter was measured 8 hrs post nucleofection. N=3. *: $p<0.05$ (Paired student's t-test).



Supplementary Figure 12

Supplementary Figure 12. The expression of HIF1α in T cell memory subsets is translationally regulated by GAPDH. Mock-transduced T_{EXP} (mock) or GAPDH-transduced (GAPDH-Td) T_{EXP} were stimulated with OKT3/a-CD28 Abs stimulated in normoxia (N) or hypoxia (H). GAPDH mRNA (**A**) and protein (**B**) expression of mock or GAPDH-Td cells determined by qPCR and western blot, respectively. N=3 for mRNA and N=2 for protein. (**C**) Detection of HIF1α mRNA expression in mock or GAPDH-Td T_{EXP} 72 hrs after stimulation. N=3. (**D**) Expression of CD45RA and CCR7 on $CD4^+$ and $CD8^+$ mock or GAPDH-TD T_{EXP} cells. N=3. (**E**) Glucose consumption at 24 hrs post-stimulation. N=3; *p=0.0194; **p=0.0017 (two-way ANOVA with post-hoc analysis). (**F**) Percentage of $Ki67^+$ mock-transduced or GAPDH-transduced T_{EXP} at 72 hrs post-stimulation. N=3; ****: p<0.0001 (two-way ANOVA with post-hoc analysis). (**G**) *In vivo* proliferation of CAR.GD2-T co-transduced with GAPDH or mock vector in normoxic ($HP1^-$) or hypoxic ($HP1^+$) tumor area. **: p=0.0025 (Two-way ANOVA with Bonferroni post-hoc analysis). Error bars indicate standard deviation.