Supplementary Figure legends

Supplementary Fig. 1 Patient-derived glioblastoma neurospheres and cell lines have different sensitivity to oHSV infection and replication. Human GBM neurospheres and cell lines were infected with oHSV-GFP at MOI=0.02 and analyzed at 16hr (**a,** representative histogram from triplicates) and 96hr (**b**) by flow cytometry (n=3) to quantify the % of GFP+ve infected cells.

Supplementary Fig. 2 PKR, but not STING or TLR signaling controls oHSV sensitivity in GBM. a. Confirmation of effectiveness of siRNA targeting PKR and STING. Shown are western blots of LN229 cells transfected with control scramble siRNA or PKR siRNA for 72hr. **b**. Representative fluorescent images of data shown in Fig 1a. LN229 cells transfected with control, STING or PKR siRNA, or treated with MyD88 inhibitor (NBP2-29328, 5μM) for 16hr and then infected with oHSV for another 72hr, MOI=0.02. **c.** Representative fluorescent microscopy (**c**) images and histogram (**d**) of GFP+ve infected U87 cells with siPKR transfection and oHSV-GFP infection at MOI=0.02. **e.** Quantification of live cell numbers after treatment with siRNA targeting PKR and infection with oHSV-GFP. Live tumor cell number was quantified by aqua live/dead staining and flow cytometry (**f**) (n=3). Error bars are s.d.,Student's t test (**p* value < 0.05).

Supplementary Fig. 3 Genomic structure of oHSV virus designed to knock down PKR in infected tumor cells. There is an insertion of shRNA targeting human PKR (oHSV-shPKR), or murine PKR (oHSV-mshPKR), or a scrambled control (oHSV-shCtl) within the ICP6 disrupted locus. Deletion of both the copies of ICP34.5 is depicted by triangles.

Supplementary Fig. 4 oHSV-shPKR increases tumor lysis in both resistant and sensitive GBM cells. LN229 cells and primary GBM cells (GBM28) were infected with oHSV-shCtl or oHSV-shPKR at MOI=0.05 (for LN229) or MOI=0.02 (for GBM28) for 72hr. Tumor cell lysis was monitored by flow cytometry analysis for live/dead staining (n=3). Error bars are s.d., Student's t test (**p* value < 0.05).

Supplementary Fig. 5 PKR regulates immune cell-mediated tumor cell lysis during oHSV treatment. oHSV sensitive GBM28 or resistant LN229 cells were infected with oHSV (MOI=0.02) in the presence or absence of PBMCs (E:T=1:5) for 96hr. Tumor cell lysis was quantified with aqua live/dead staining (**a-b**).(n=3). Error bars are s.d., Student's t test (**p* value < 0.05).

Supplementary Fig. 6 oHSV-shPKR increases CD8 T cell activation and IFNγ secretion during immune cell-mediated tumor cell lysis. GSC20 cells were infected with oHSV-shCtl or oHSV-shPKR (MOI=0.02) and overlaid with or without PBMCs for 72hrs. CD8 T cell activation was analyzed by CD69

floy cytometry staining (**a**) (n=3). IFNγ secretion in the co-culture was analyzed by ELISA (**b**) (n=3). Error bars are s.d., Student's t test (**p* value < 0.05).

Supplementary Fig. 7 oHSV-shPKR increases activity of antigen presenting cells. PBMC-derived DCs cultured with GSC20 cells treated with oHSV-shPKR or oHSV-shCtl were analyzed for markers indicative of activation. A-b: representative dot blots and mean MFI of CD86 and HLADR on dendritic cells, ± s.d (**b,** n=3/g) analyzed by flow cytometry. The apoptosis of dendritic cells was analyzed by Annexin-V/PI staining (**c-d,** representative dot plot**, n=3**). Error bars are s.d., Student's t test (**p* value < 0.05).

Supplementary Fig. 8 oHSV-shPKR increases antigen-specific cytotoxic T lymphocyte activation. Representative dot blots of data quantified in Fig 3. Autologous T cells cultured with dendritic cells charged with treated gbm cell conditioned medium (as shown in in Fig.3c) were analyzed for activation markers by flow cytomentry. **a**. dot blots of Flow cytometry analysis of CD69 on $CD4^+$ and $CD8^+$ T-cells cultured with dendritic cells. **b-c**. dot blots of Flow cytometry analysis of intracellular staining of effector molecules IFNγ and TNFα in both $CD8^+$ (**b**) and $CD4^+$ (**c**). **d**. Anti-GBM and anti-viral specific CTLs were analyzed by EphA2-tetramer and HSV gB-tetramer staining. Flow cytometry dot plots are showed from one of triplicates.

Supplementary Fig. 9 Tumor cell antigen release from infected GBM cells. Western blot analysis of tumor antigen EphA2 in 5× concentrated supernatant (20ul) from GSC20 and U251T3 cells infected with oHSV-shCtl or oHSV-shPKR.

Supplementary Fig. 10 oHSV-mshPKR murine specific PKR regulating oHSV. **a.** Three siRNAs against mouse PKR were designed and transfected into mouse glioma cells NP for 16hr and then infected with oHSV (MOI=0.02) for 72hr. The viability of tumor cells was analyzed by cell-titer glo assay (n=3). (b) PKR knockdown by oHSV-mshPKR in GL261N4 glioma cells. **b.** Murine siPKR3 sequence was used to construct oHSV-mshPKR. GL261N4 cells were infected with oHSV-mshPKR or oHSV-shCtl (MOI=1) for 48hr. Murine PKR knockdown was analyzed by qRT-PCR (n=3). **c-e.** GL261N4 and DB7 cells were infected with oHSV-mshPKR or oHSV-shCtl (MOI=0.02) for 96hr. oHSV-infected GBM cells were quantified by flow cytometry. Representative histograms are showed (**c**). Tumor cell lysis by oHSV-mshPKR in GL261N4 and DB7 cells was analyzed by Near-IR live/dead staining (**d-e**) (n=3). Error bars are s.d., Student's t test (**p* value < 0.05).

Supplementary Fig. 11 Single-cell RNA-seq of myeloid cells in GL261N4 tumor bearing mice treated with oHSV-mshPKR. Murine GL261N4 tumors implanted in C57BL/6 mice were treated with oHSV-mshPKR or oHSV-shCtl. 5 days after treatment, CD45+ cells and CD45- cells harvested from tumor-bearing mice underwent scRNA-seq analysis (n=5 mice/group). Annotation of

myeloid cell populations **(a)** and different cell types **(b)** within the myeloid cell population from tumor-bearing mice were assessed.

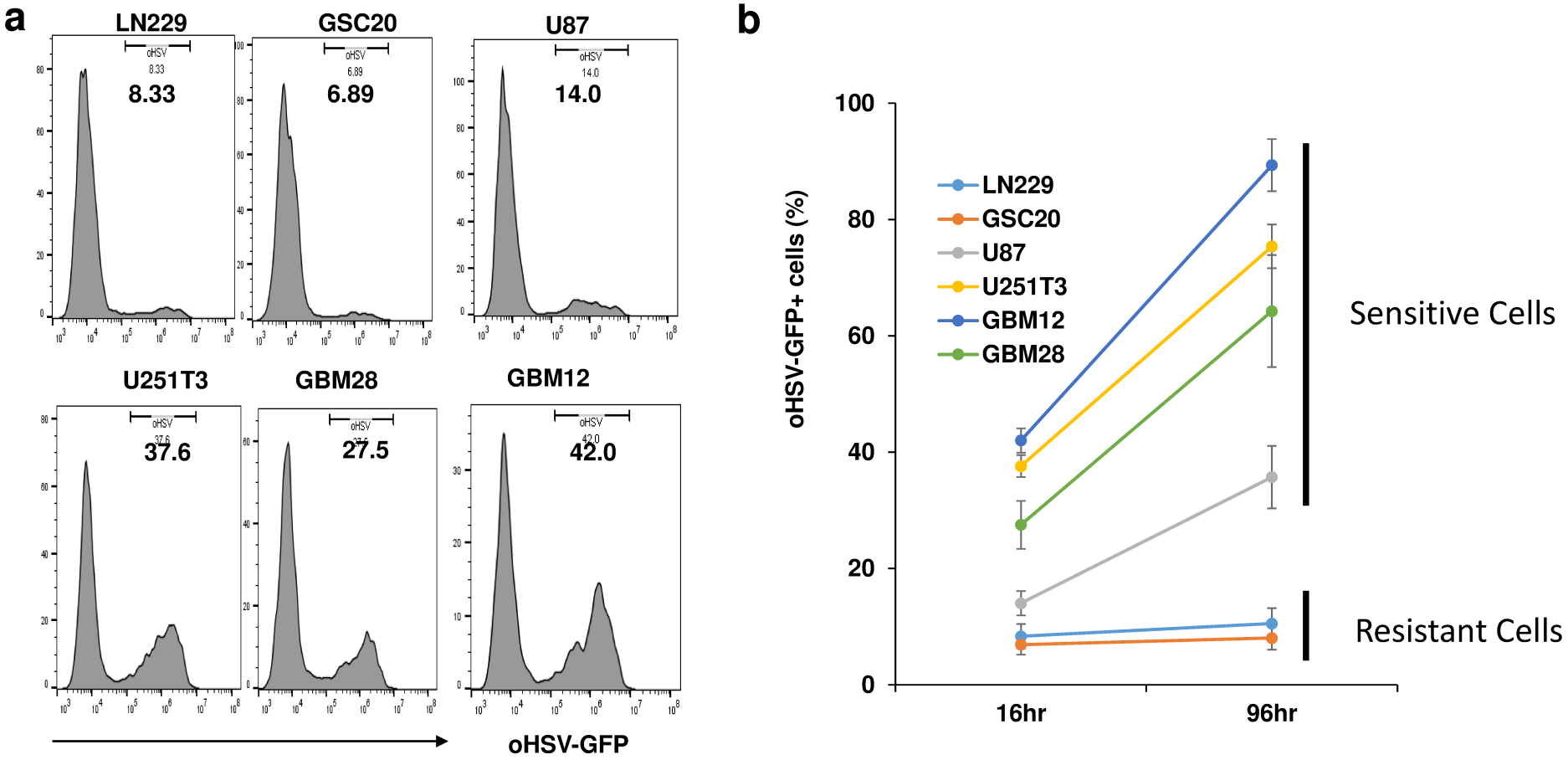
Supplementary Fig. 12 Single-cell RNA-seq analysis of TGFβ signaling in different cell types found in human glioblastoma. Analysis of TGFβ signaling in different cell types from scRNA-seq from a total of 3589 cells from 4 human GBM patients. (The raw scRNA-seq data are from Gephart Lab: Darmanis, S., Sloan, SA., Croote, D., Mignardi, M., Chernikova, S., Samghababi, P., Zhang, Y., Neff, N., Kowarsky, M., Caneda, C., Li, Gordon., Chang, S., Connolly, I.D., Li, Y., Barres, B., Gephart, M.H., Quake, S.R. Single-Cell RNAseq analysis of infiltrating neoplastic cells at the migrating front of human glioblastoma. 2017)

Supplementary Fig. 13 TGFβ secretion from GBM28 cells after infection of oHSV-shPKR. GBM28 cells were infected with oHSV-shPKR or oHSV-shCtl (MOI=0.05) for 48hr. TGFβ secretion was analyzed by ELISA (n=3). Error bars are s.d., Student's t test (**p* value < 0.05).

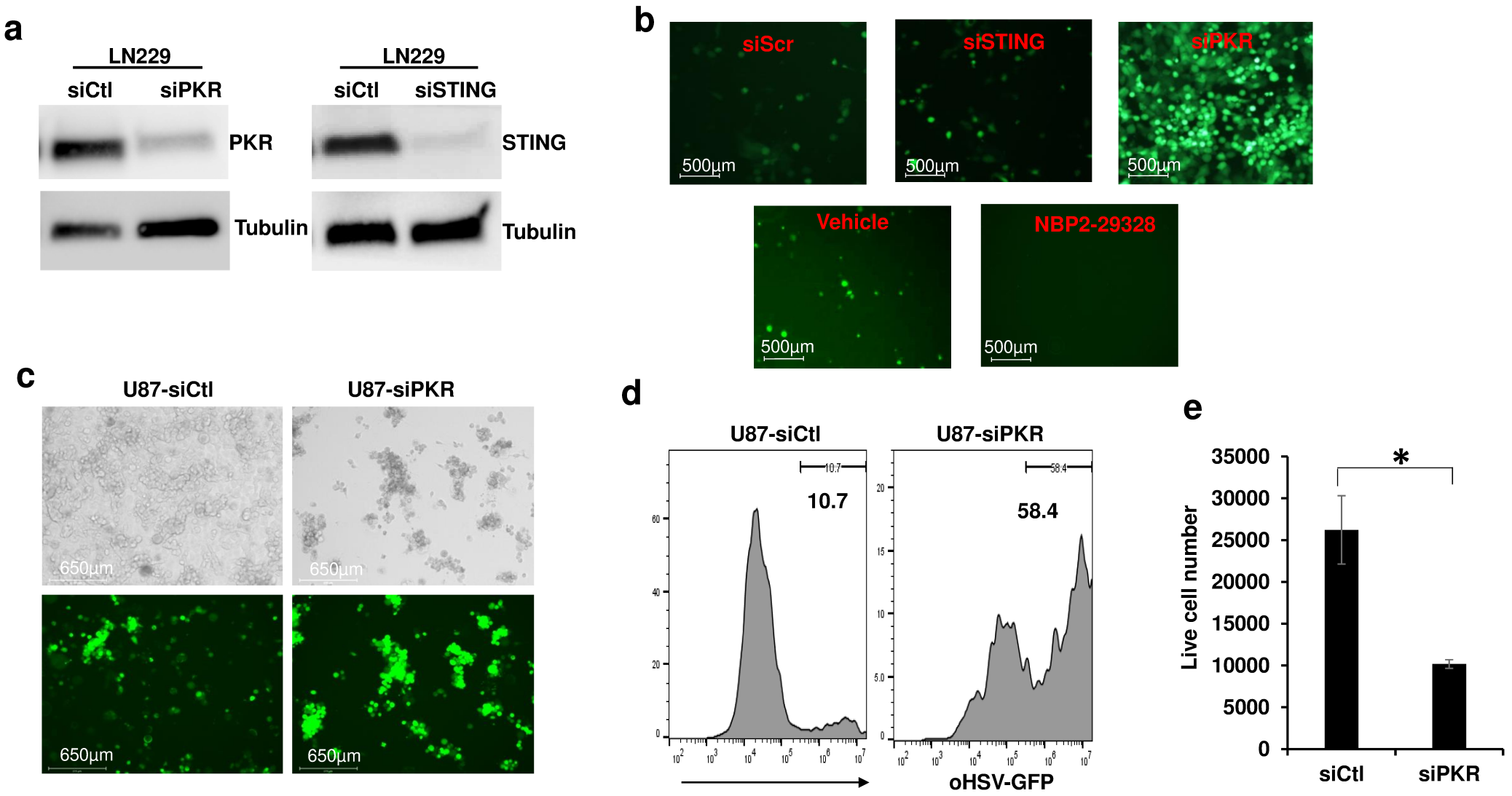
Supplementary Fig. 14 oHSV-mshPKR increases antigen specific T cells expansion. a. GL261N4-OVA cells were infected with oHSV-mshPKR or oHSV-shCtl (MOI=0.05) for 24hr and then cocultured with OT-1 T cells (1:5) for 72hr. Flow cytometry analysis of live tumor cells by aqua live/dead staining gating on tumor cells only (n=3). **b-c.** PD1 expression in the cocultured CD8 T cells were analyzed by flow cytometry (n=3)**.** Error bars are s.d., Student's t test (**p* value < 0.05).

Supplementary Fig. 15 oHSV-mshPKR increases MHC class I-bound SIINFEKL peptide from GL261N4-OVA cells. GL261N4-OVA cells were infected with oHSV-shCtl or oHSV-mshPKR with MOI=0.5 for 72hr. MHC class I-bound SIINFEKL peptide in GL261N4-OVA cells was analyzed flow cytometry (n=3). Error bars are s.d., Student's t test (**p* value < 0.05).

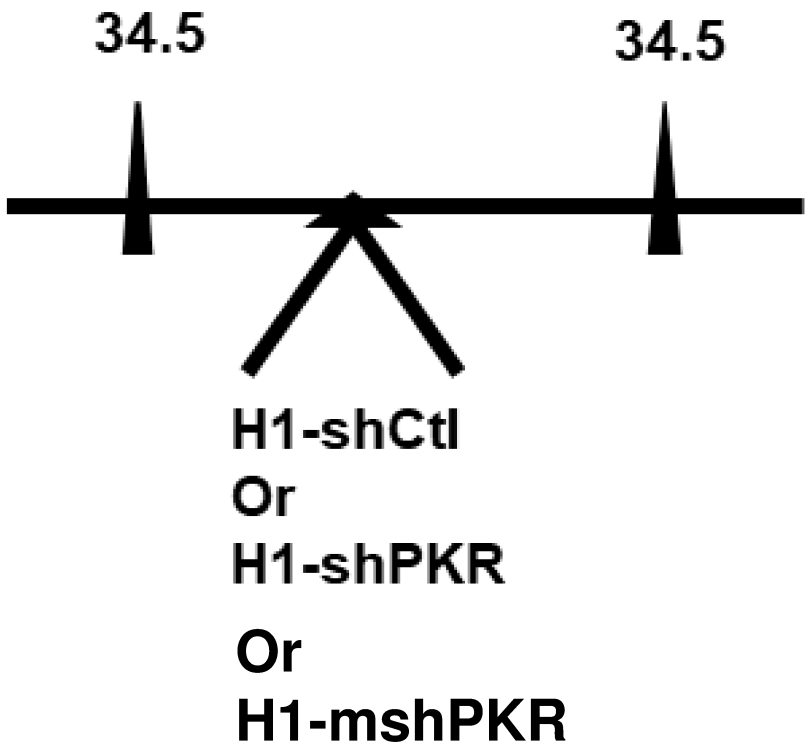
Supplementary Table.1 siRNA and shRNA sequence used in the study.



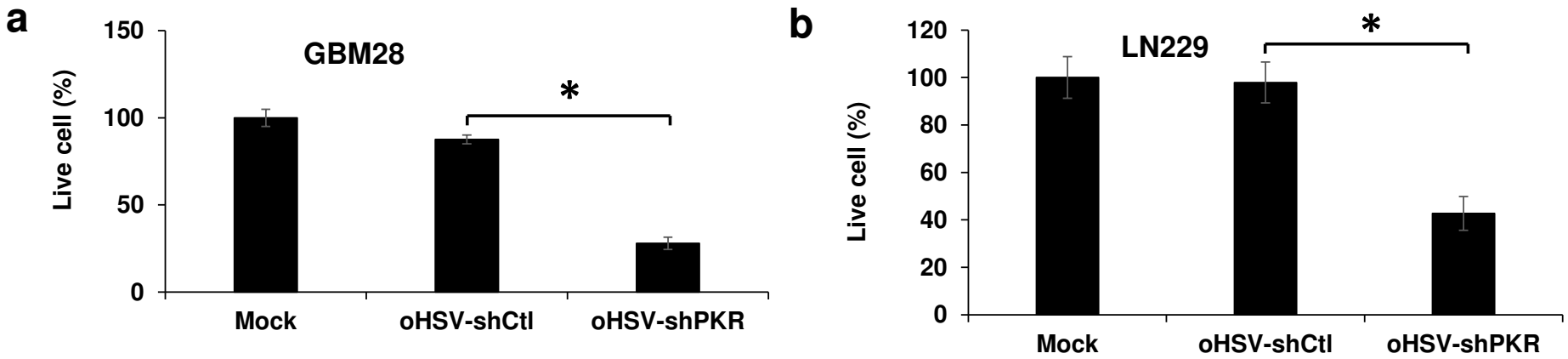
Supplementary Fig. 1



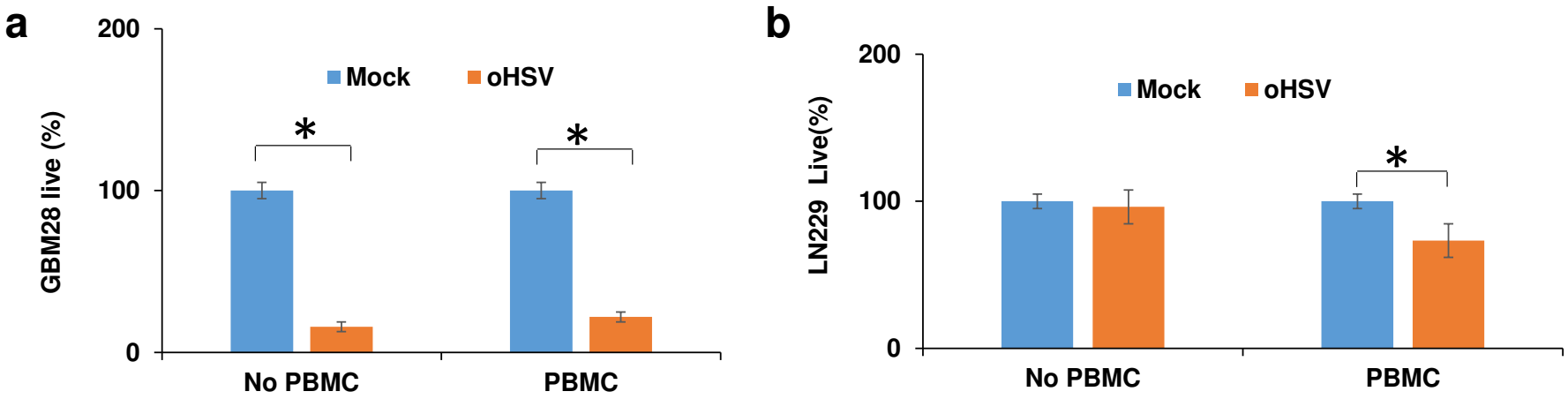
Supplementary Fig. 2



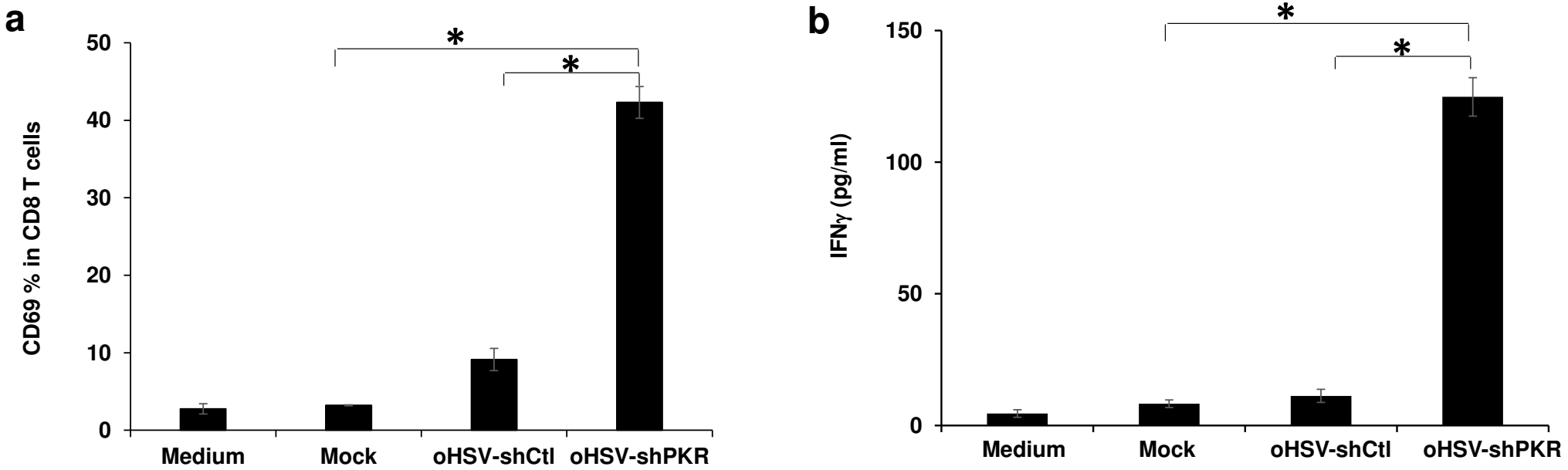
Supplementary Fig. 3



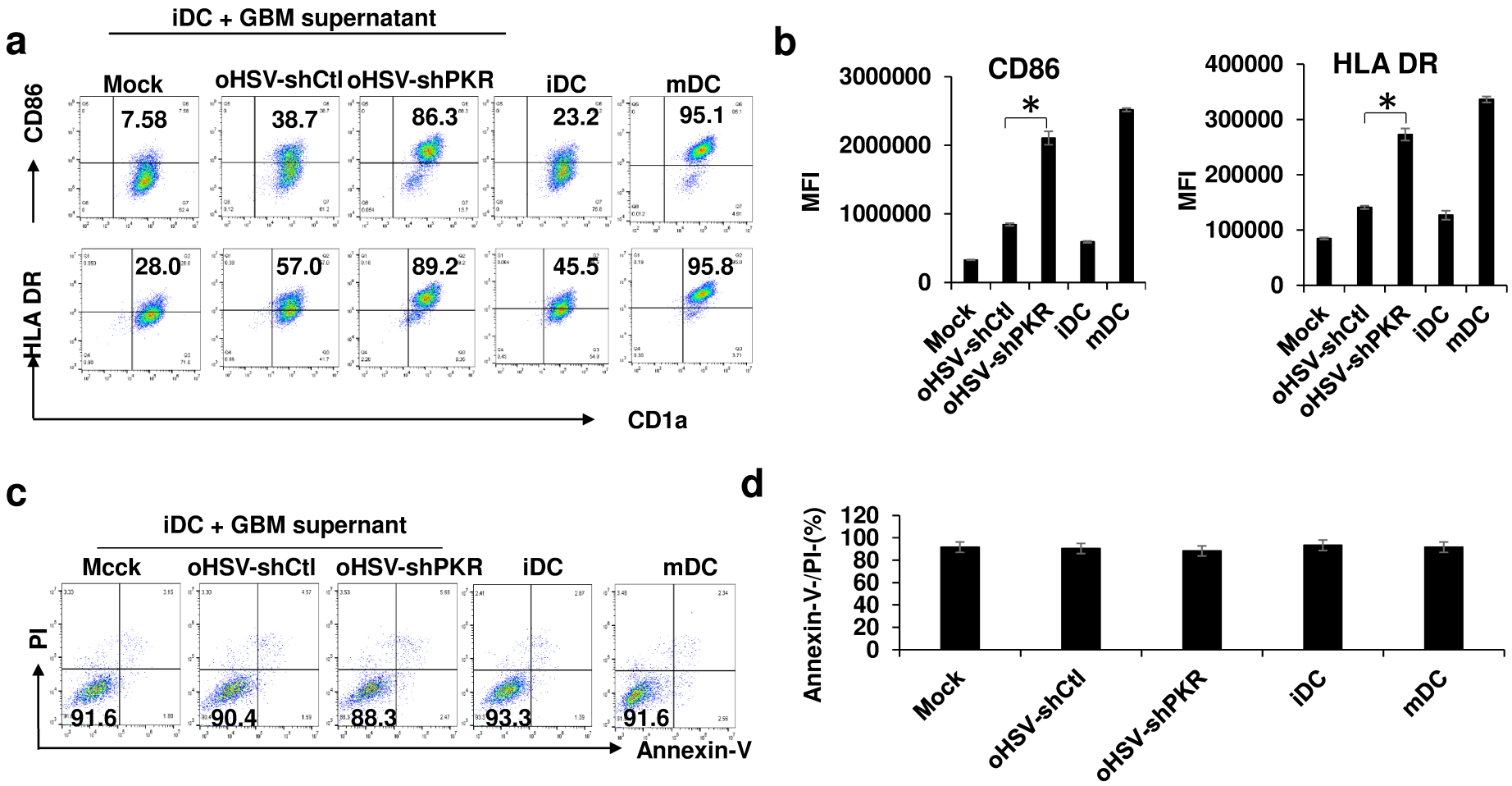
Supplementary Fig. 4



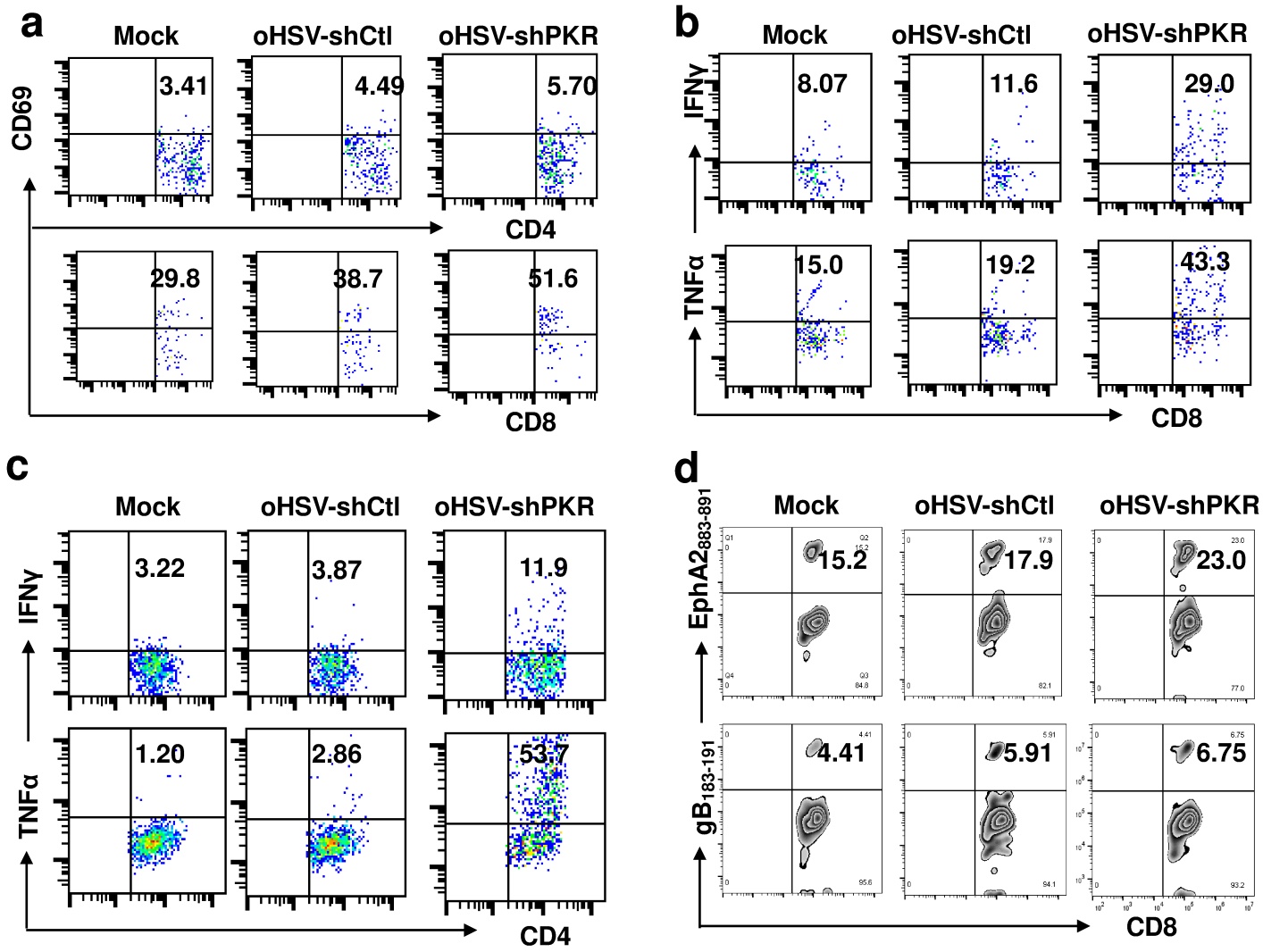
Supplementary Fig. 5



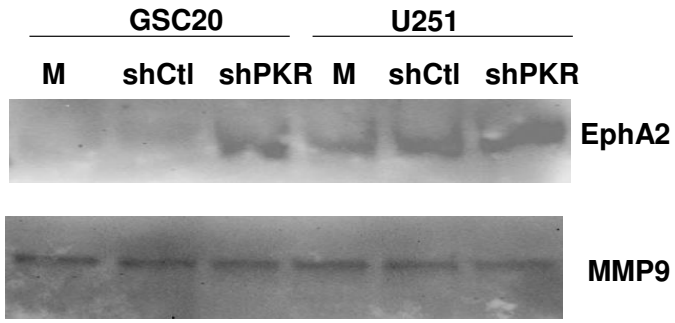
Supplementary Fig. 6



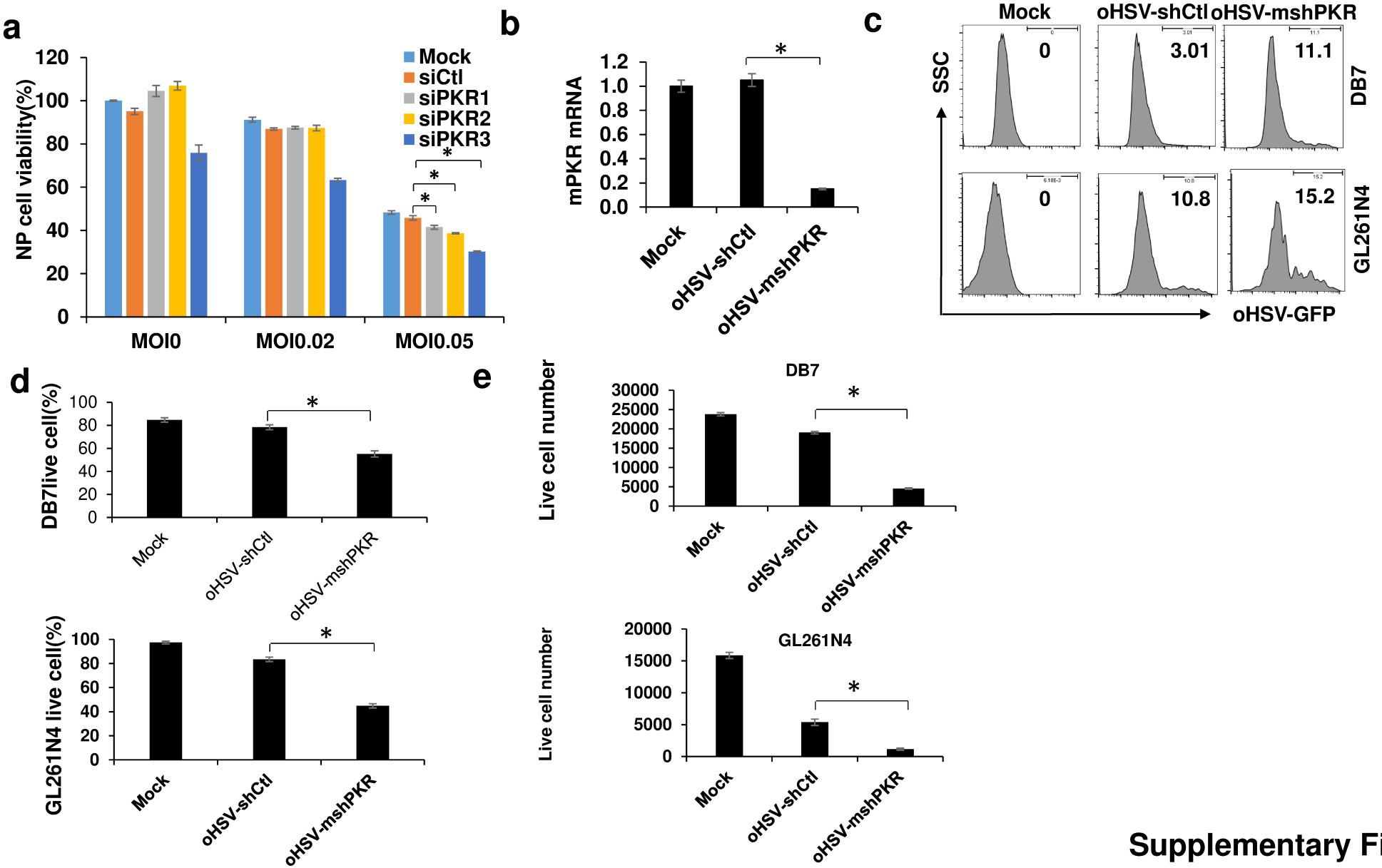
Supplementary Fig. 7



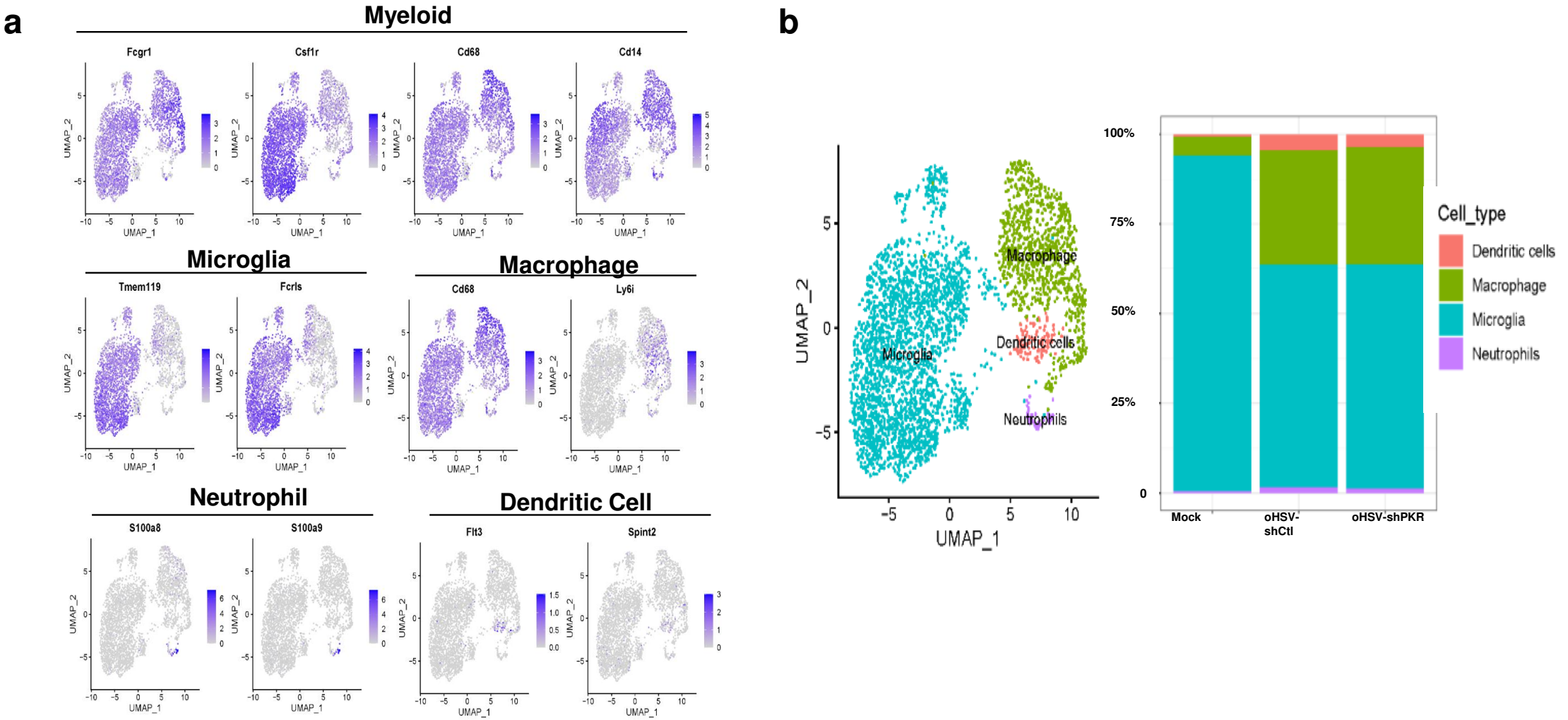
Supplementary Fig. 8



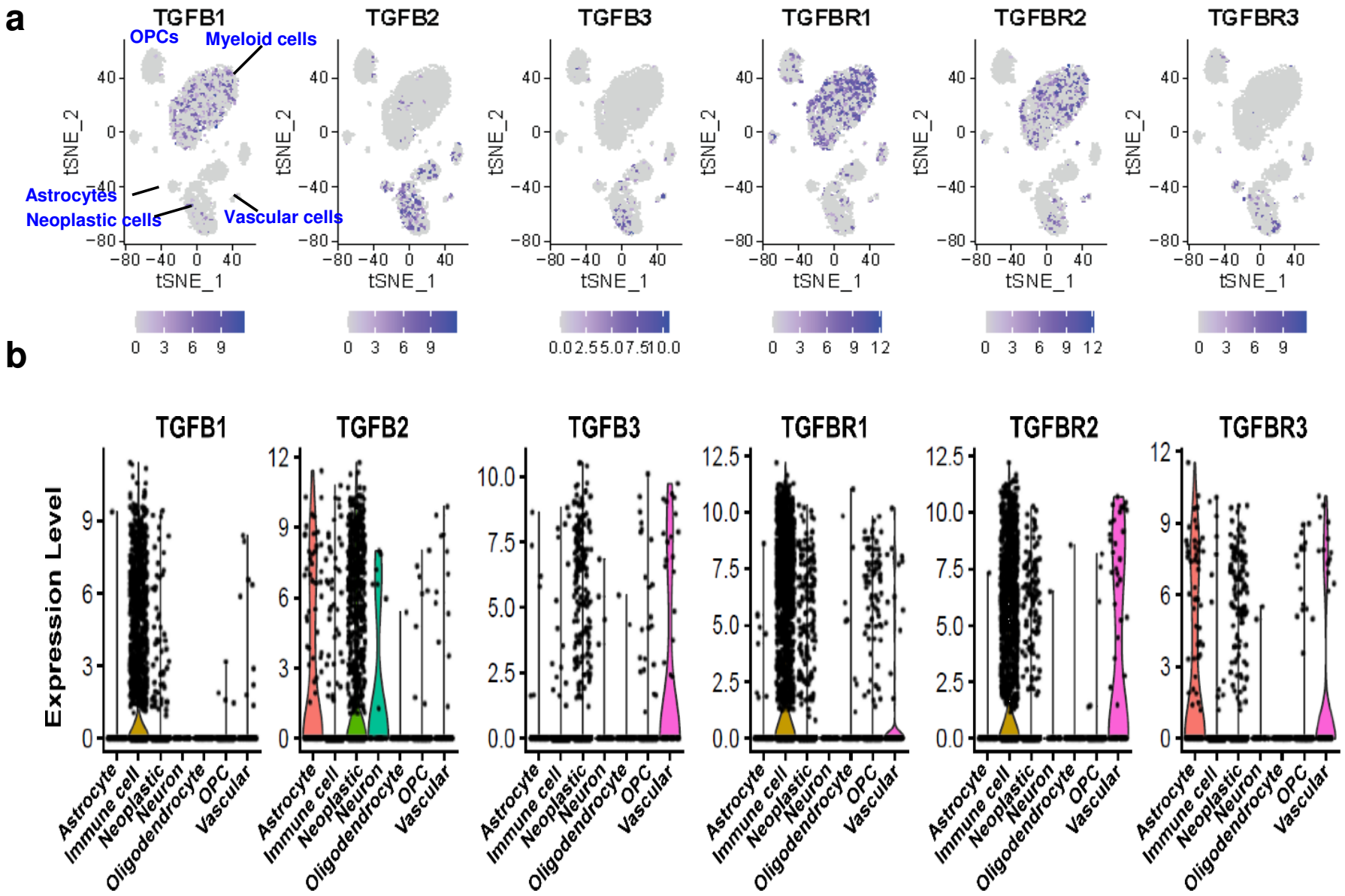
Supplementary Fig. 9



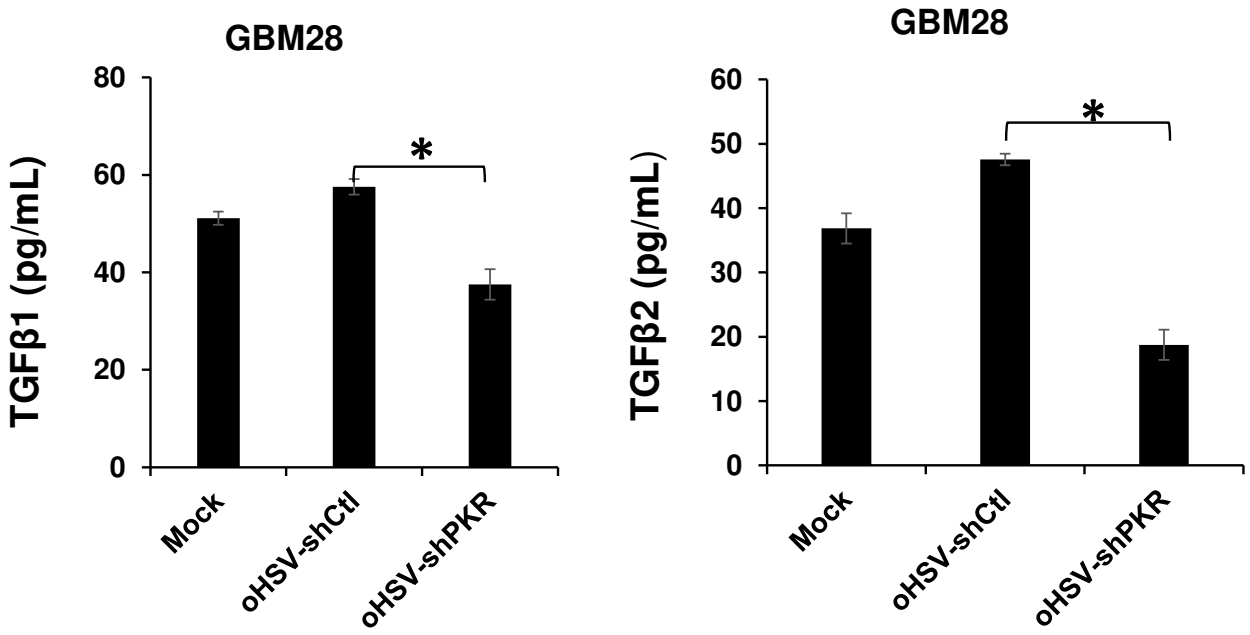
Supplementary Fig. 10



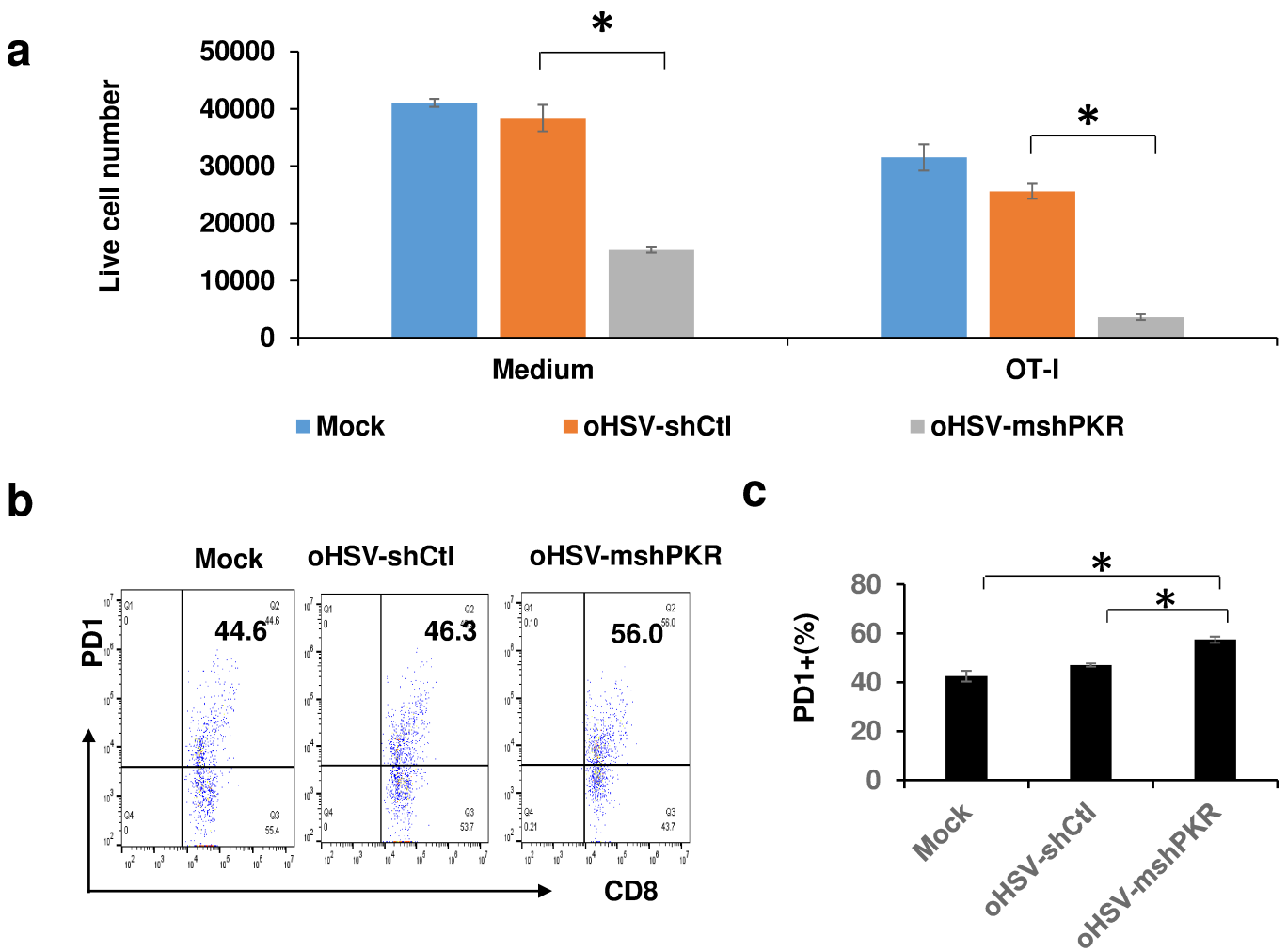
Supplementary Fig. 11



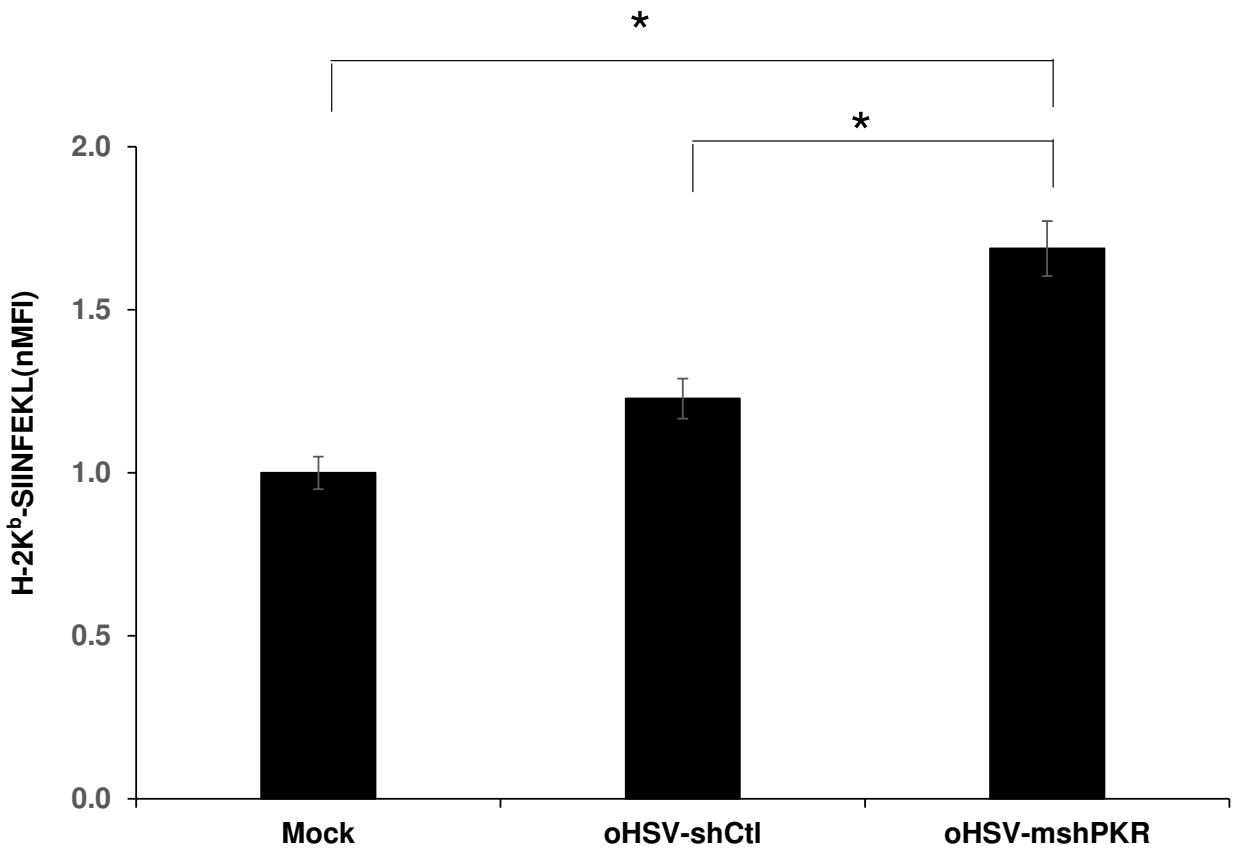
Supplementary Fig. 12



Supplementary Fig. 13



Supplementary Fig. 14



Supplementary Fig. 15

Supplementary Table 1. siRNA and shRNA sequence used in the study

siRNA or shRNA	Sequence
Scramble siRNA	5’-UAAGGCUAUGAAGAGAUAC-3’
Human PKR siRNA1	5’-CAUCAGAGAUAAAUUCUAA-3’
Human PKR siRNA2	5’-GUCAGAAGCAGGGAGUAGU-3’
Murine PKR siRNA1	5’-CAAAGCAGUUGGCUGCGAA-3’
Murine PKR siRNA2	5’-GAAGGUUUACAUUUCAAGU-3’
Murine PKR siRNA3	5’-GUGAUACAAGUCGAUACAA-3’
Scramble shRNA	5’-TAAGGCTATGAAGAGATACttcaagagaGTATCTCTTCATAGCCTTA-3’
Human PKR shRNA1	5’-CATCAGAGATAAATTCTAAttcaagagaTTAGAATTTATCTCTGATG-3’
Human PKR shRNA2	5’-GTCAGAAGCAGGGAGTAGTttcaagagaACTACTCCCTGCTTCTGAC-3’
Murine PKR shRNA1	5’- CAAAGCAGTTGGCTGCGAA ttcaagagaTTCGCAGCCAACTGCTTTG-3’
Murine PKR shRNA2	5’-GAAGGTTTACATTTCAAGTttcaagagaACTTGAAATGTAAACCTTC-3’
Murine PKR shRNA3	5’-GTGATACAAGTCGATACAAttcaagagaTTGTATCGACTTGTATCAC-3’