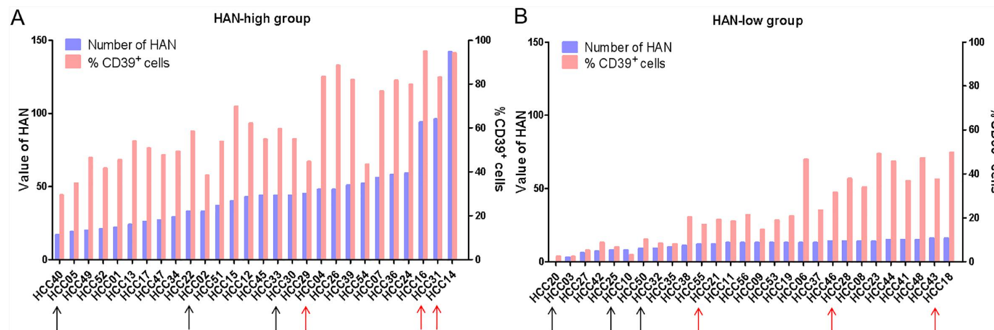
1 # Supplemental Figures and legends



2

3 **Supplemental Figure 1**

4 Summary of the frequency of $CD39^{+}CD8^{+}$ TILs and value of HAN for all the 56 HCC

5 patients. (A-B) Red bars represented the frequency of $CD39^{+}CD8^{+}$ TILs and blue bars

6 represented the value of HAN. Arrows indicated the six representative patients

7 selected for TILs and autologous tumors organoids killing assay. Red arrows indicated

8 three candidate patients selected for specific high affinity peptide identification assay.

	patients ID	HLA-class I		
HAP-high group	HCC14	HLA-A*02:06 HLA-A*33:01	HLA-B*15:02 HLA-B*37:01	HLA-C*03:02 HLA-C*08:01
	HCC31	HLA-A*11:01 HLA-A*31:01	HLA-B*13:01 HLA-B*46:01	HLA-C*01:02 HLA-C*03:04
	HCC04	HLA-A*02:03 HLA-A*31:01	HLA-B*39:03 HLA-B*40:01	HLA-C*03:04 HLA-C*07:02
HAP-low group	HCC18	HLA-A*02:01 HLA-A*11:02	HLA-B*46:01	HLA-C*01:02
	HCC28	HLA-A*02:07 HLA-A*24:02	HLA-B*35:01 HLA-B*51:01	HLA-C*03:03 HLA-C*14:02
	HCC21	HLA-A*11:01 HLA-A*24:02	HLA-B*27:04 HLA-B*40:01	HLA-C*03:04 HLA-C*12:02

9

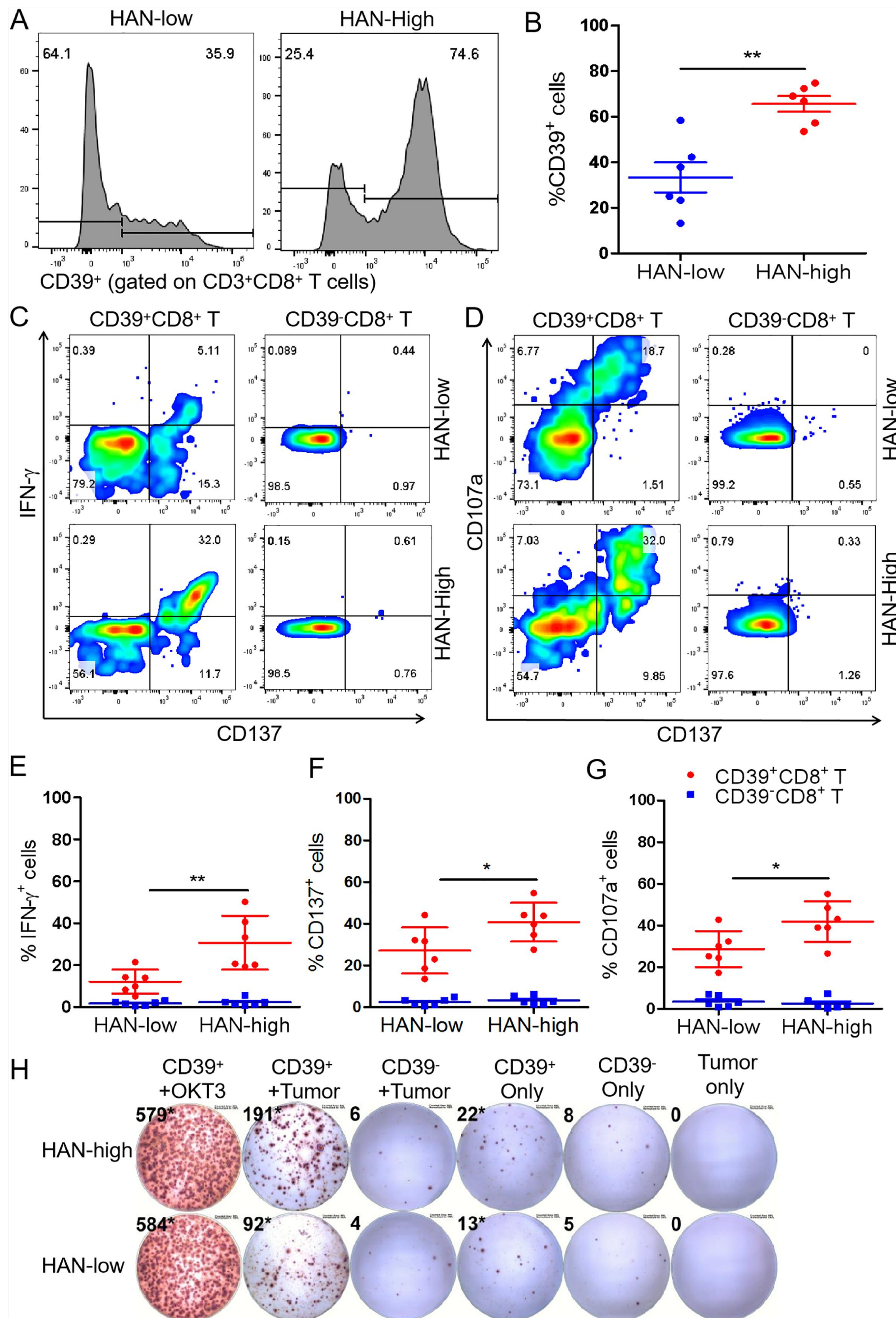
10 **Supplemental Figure 2**

11 HLA alleles Analysis by OptiType(v1.3.3) were shown for six candidate patients. (A)

12 The coverage plot of the HLA alleles for representative patient HCC14 analyzed by

13 OptiType were shown. Listed HLA alleles were with high expression abundance.

14 (B) Summary HLA alleles of all the six candidate patients were shown in this table.



15

16 **Supplemental Figure 3**

17 CD39 and effector molecules expressed on TILs from surgically removed HCC fresh

18 tumor samples. (A-B) The frequency of $CD39^{+}CD8^{+}$ T cells in each group was

19 detected by flow cytometry. (C-G)The expression of IFN-γ, CD107a and CD137 on

20 $CD39^{+/-}CD8^{+}$ T cells was detected by flow cytometry. The dots represent different

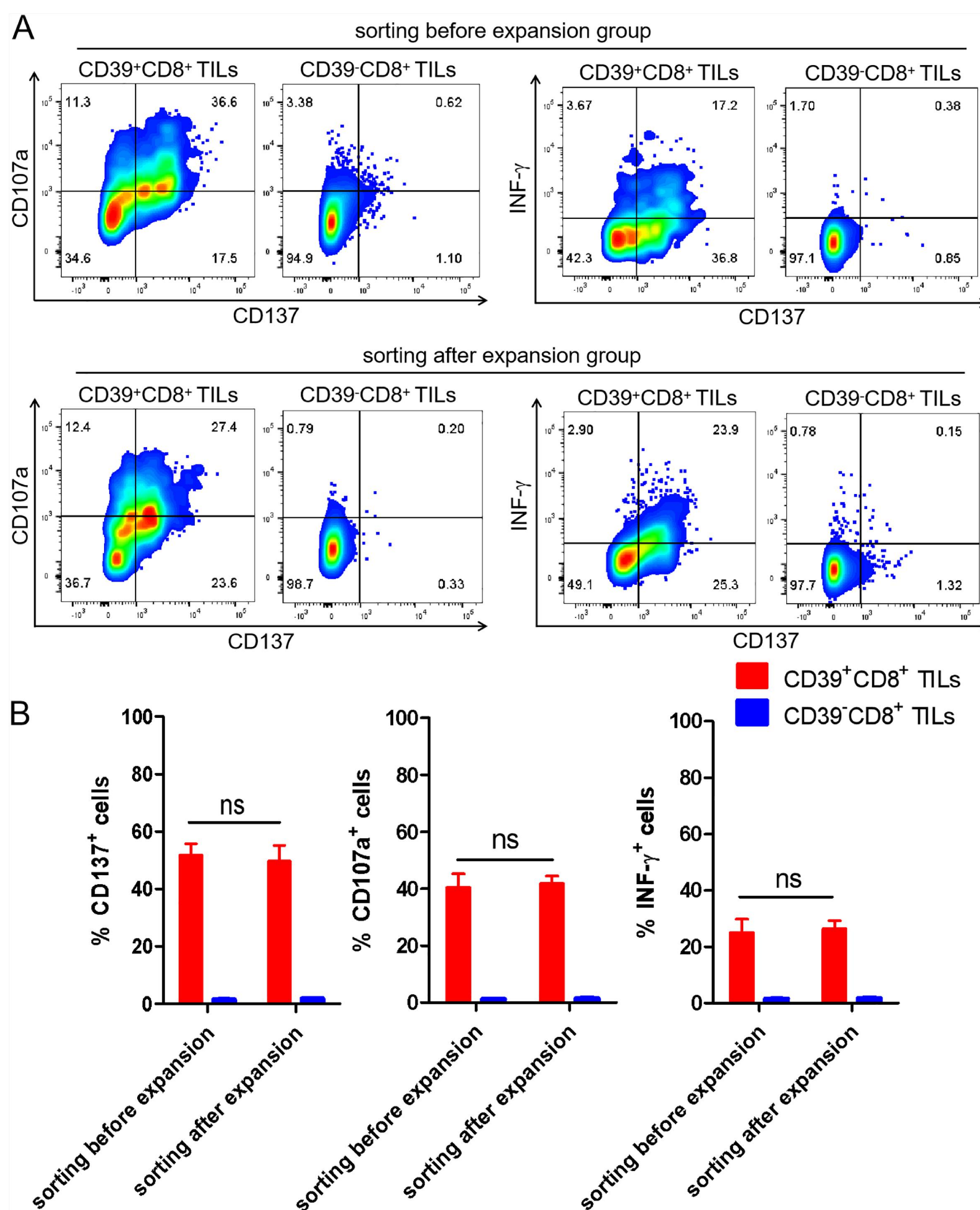
21 patients. Data are presented as mean ± SEM (n=6), $*P< 0.05$, $**P < 0.01$.(H) $CD39^{+/-}$

22 $CD8^{+}$ TILs were respectively sorted from surgically removed fresh tumor sample and

23 co-cultured with or without (TILs only) autologous tumor cells, then the IFN-γ

24 secretion was investigated by ELISPOT after 24 hours. OKT3 was used as positive

25 control.

26 

27 **Supplemental Figure 4**

28 The IFN-γ secretion, CD137 and CD107a expression on $CD39^{+/-}$ TILs had no bias in

29 expansion before sorting group and expansion after sorting group. (A-B) TILs were

30 isolated from surgically removed HCC fresh tumor sample and divided into two

31 groups. The one was the sorting before expansion group which TILs were sorted

32 immediately and then $CD39^{+/-}$ TILs were expanded respectively for 14 days. Another

33 one was the sorting after expansion group which TILs were expanded first for 14 days

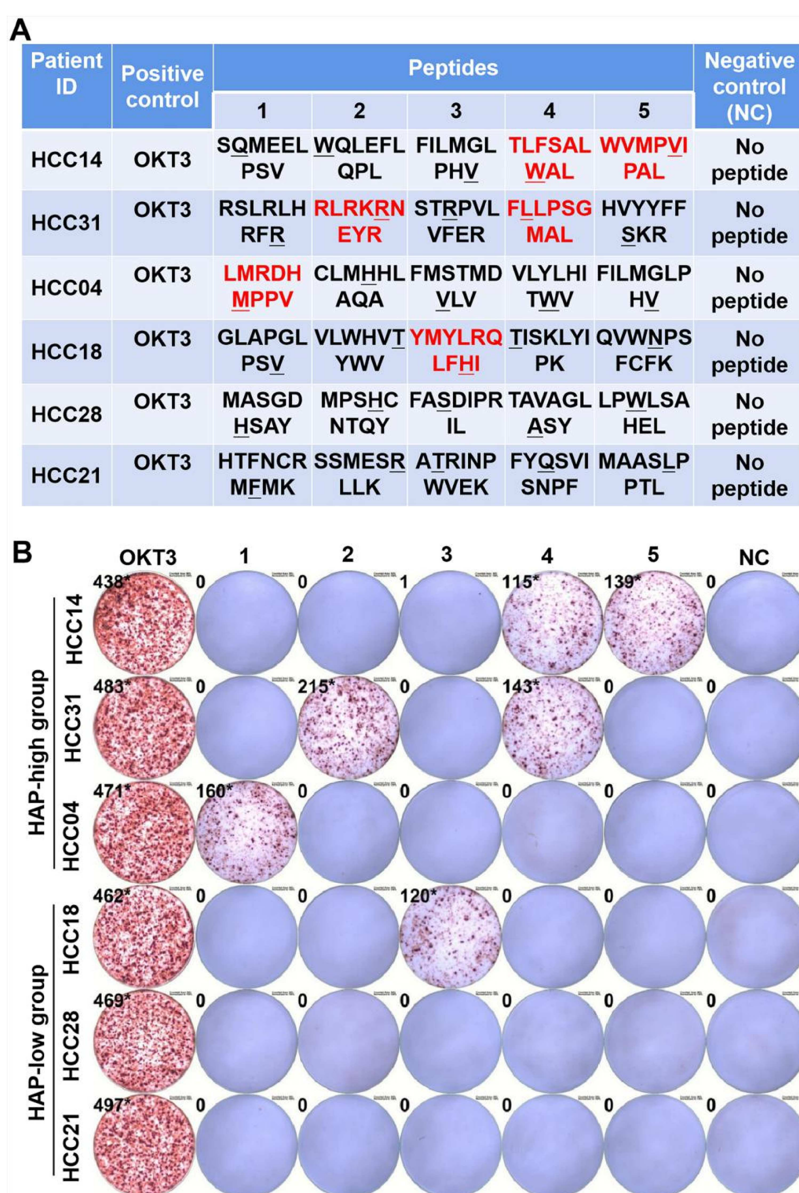
34 and then sorted by the marker of CD39. At day 14, TILs of each group was

35 co-cultured with tumor cells, and then the function of CD39$^{+/-}$ subsets was assessed by

36 flow cytometry with intracellular staining of IFN-γ, CD107a and CD137. (B)

37 Summary of the frequency of IFN-γ, CD107a and CD137 positive was shown.

38

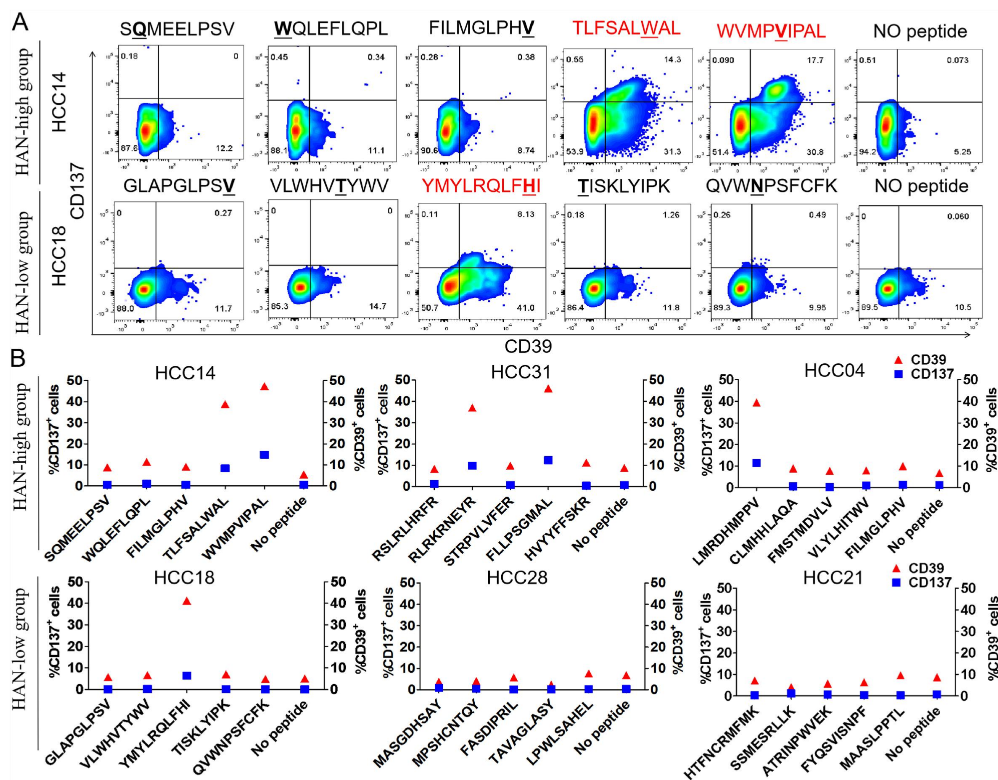


A

Patient ID	Positive control	Peptides					Negative control (NC)
		1	2	3	4	5	
HCC14	OKT3	SQMEEL PSV	WQLEFL QPL	FILMGL PHV	TLFSAL WAL	WVMPVI PAL	No peptide
HCC31	OKT3	RSLRLH RFR	RLRKRN EYR	STRPVL VFER	FLLPSG MAL	HVYYFF SKR	No peptide
HCC04	OKT3	LMRDH MPPV	CLMHHL AQA	FMSTMD VLV	VLYLHI TWV	FILMGLP HV	No peptide
HCC18	OKT3	GLAPGL PSV	VLWHVT YWV	YMYLRQ LFHI	TISKLYI PK	QVWNPS FCFK	No peptide
HCC28	OKT3	MASGD HSAY	MPSHC NTQY	FASDIPR IL	TAVAGL ASY	LPWLSA HEL	No peptide
HCC21	OKT3	HTFNCR MFMK	SSMESR LLK	ATRINP WVEK	FYQSVI SNPF	MAASLP PTL	No peptide

39 **Supplemental Figure 5**

40 Identification of personalized neoantigens for 6 patients. (A-B) Autologous PBMCs
41 were stimulated with top five candidate peptides for 10 days, after which autologous
42 tumor organoid from candidate patient was added to activate T-cell specific antigen
43 response 24h before IFN-γ detection by ELISPOT assays. OKT3 was used as positive
44 control, and no-peptide stimulation was tested as negative control.

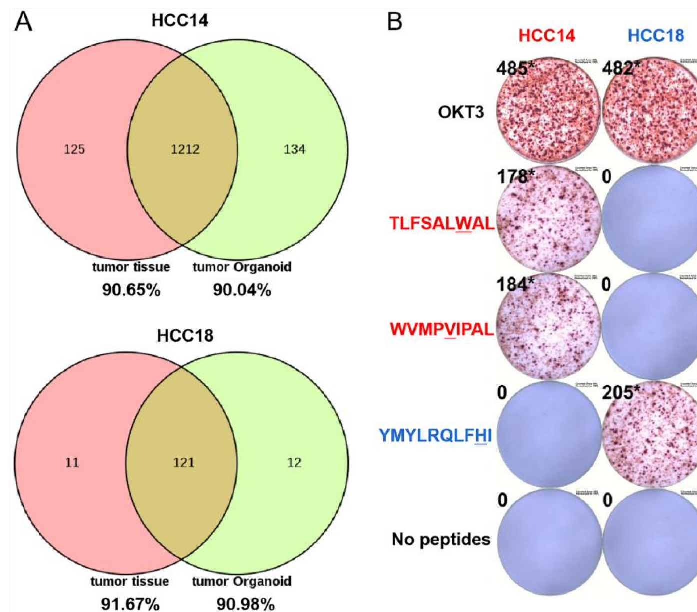


45

46 **Supplemental Figure 6**

47 Expression of CD137 and CD39 of tumor-reactive $CD39^+CD8^+$ T cells by stimulation
48 with candidate HANs from PBMCs. (A) Flow cytometry plots gated on $CD8^+$ T cells
49 to analyze T cell effector sensitivity against tumor organoids by CD137 and CD39
50 up-regulation. (B) Summary of the frequency of $CD39^+$ or $CD137^+$ T cells was shown

51 for all the six representative patients.

52 

53 **Supplemental Figure 7**

54 Allogeneic positive peptides couldn't induce IFN-γ release for autologous tumor

55 organoid killing. (A)Venn diagrams illustrate the number of somatic non-synonymous

56 mutations present in each tumor tissue and their derivative HCC organoids. The

57 similarity of mutation from tumor tissue and organoid was greater than 90%. (B)

58 Autologous PBMCs from candidate patients were stimulated with autologous peptides

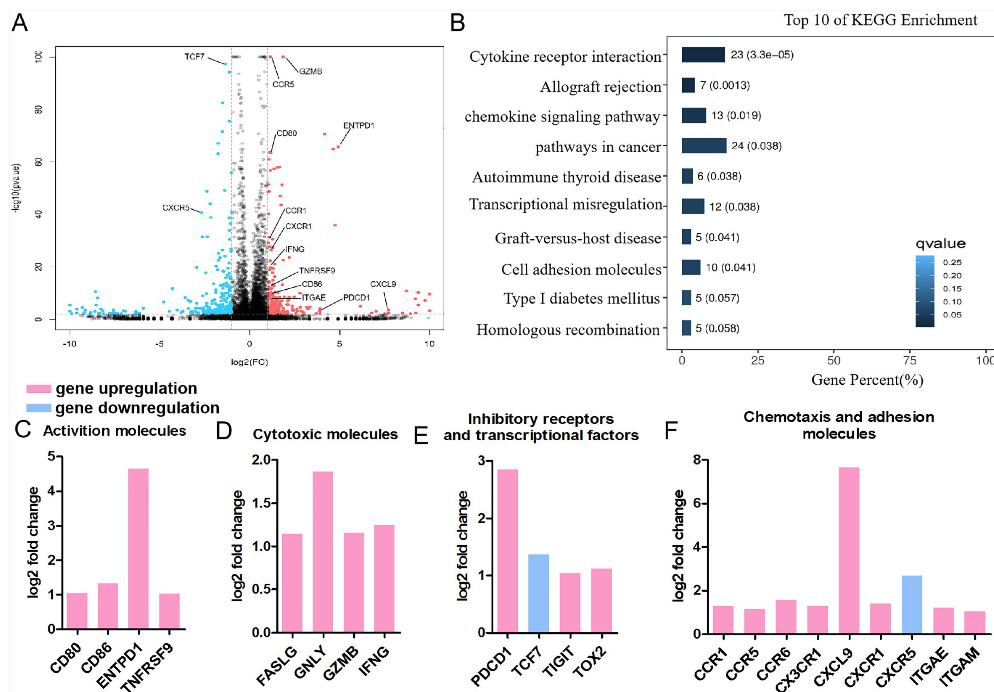
59 (marked by the same color) and allogeneic peptides (marked by the different color)

60 for 10 days, after which autologous tumor organoids from candidate patient was

61 added to activate T-cell specific antigen response 24h before IFN-γ detection by

62 ELISPOT assays. OKT3 was used as positive control, and no-peptide stimulation was

63 tested as negative control.



64

65 **Supplemental Figure 8**

66 RNA-seq data of CD39$^{+/-}$CD8^{+} T cells stimulated by HAN peptide. PBMCs were

67 stimulated by autologous HANs for 10 days and then co-cultured with autologous

68 tumor organoids for 24h. After which CD39$^{+/-}$CD8^{+} T cells were sorted by FACS for

69 following RNA-seq assay. (A) Volcano graph revealed the difference of genes

70 expression analyzed by RNA-seq between CD39$^{+/-}$ CD8^{+} T cells after stimulation by

71 HAN peptide (log2-transformed). The dashed line identified the differently expressed

72 genes when using a P value <0.05. (B) KEGG enrichment demonstrated the

73 significant enrichment of top 10 pathways using a P/Q value. (C-F) Bar plots

74 indicated the RNA-seq expression of log2 fold change of major up-regulated and

75 down-regulated genes in CD39^{+}CD8^{+} T cells compared to CD39^{-}CD8^{+} T cells (*P*

76 <0.05). Pink bars represented up-regulation and blue bars represented

77 down-regulation.