LIMIT is an immunogenic IncRNA in cancer immunity and immunotherapy

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Major histocompatibility complex-I (MHC-I) presents tumour antigens to CD8+ T cells and triggers anti-tumour immunity. Humans may have 30,000–60,000 long noncoding RNAs (lncRNAs). However, it remains poorly understood whether lncRNAs affect tumour immunity. Here, we identify a lncRNA, IncRNA inducing MHC-I and immunogenicity of tumour (LIMIT), in humans and mice. We found that IFNγ stimulated LIMIT, LIMIT cis-activated the guanylate-binding protein (GBP) gene cluster and GBPs disrupted the association between HSP90 and heat shock factor-1 (HSF1), thereby resulting in HSF1 activation and transcription of MHC-I machinery, but not PD-L1. RNA-guided CRISPR activation of LIMIT boosted GBPs and MHC-I, and potentiated tumour immunogenicity and checkpoint therapy. Silencing LIMIT, GBPs and/or HSF1 diminished MHC-I, impaired antitumour immunity and blunted immunotherapy efficacy. Clinically, LIMIT, GBP- and HSF1-signalling transcripts and proteins correlated with MHC-I, tumour-infiltrating T cells and checkpoint blockade response in patients with cancer. Together, we demonstrate that LIMIT is a cancer immunogenic IncRNA and the LIMIT–GBP–HSF1 axis may be targetable for cancer immunotherapy.
**Fig. 1 | LIMIT is an immunogenic IncRNA.** a, Human melanoma samples (TCGA dataset, SKCM, n = 472 patients) were divided into hot and cold tumours on the basis of CD8A transcripts. The volcano plots show the fold changes and P values of 3,926 IncRNA candidates in hot tumours (CD8A, top 10%) versus cold tumours (CD8A, bottom 10%). Statistical analysis was performed using two-sided t-tests. b–d, Correlation of LIMIT with IFNG (b), MHC-I (c) or CD8A (d) in patients with melanoma (TCGA, SKCM). Statistical analysis was performed using two-sided linear regression. e–h, Human melanoma samples (TCGA, SKCM) were divided into high (n = 236 patients) and low (n = 236 patients) LIMIT tumours. GSEA showed the indicated gene signatures. The following gene signatures were enriched in high LIMIT tumours: response to IFNγ (the bottom 15% versus top 15%). The response rates to immune checkpoint blockade were calculated as the percentages of partial response (PR) plus complete response (CR). Statistical analysis was performed using χ² tests. Patients were from four cohorts. j, Survival plot of patients with melanoma (TCGA, SKCM). Patients were divided into high (n = 236 patients) and low (n = 236 patients) LIMIT groups. Statistical analysis was performed using two-sided log-rank tests. k, A375 cells were treated with the indicated cytokines (5 ng ml⁻¹) for 24 h. LIMIT was detected using northern blotting (NB). The 28S rRNA, 18S rRNA and 5S rRNA are shown as loading controls. One out of two experiments is shown. l, m, 5’RACE and 3’RACE analysis of human LIMIT (l) or murine Limit (m). One out of two experiments is shown. n, A375 cells were treated with IFNy for 24 h. LIMIT was detected using RT-PCR in nuclear or cytoplasmic RNAs. Unspliced ACTB and mature ACTB were used as controls for nuclear and cytoplasmic RNAs, respectively. One out of two experiments is shown. o, Reads per kb of transcript per million mapped reads (RPKM) of LIMIT in different cancer cells in response to IFNy. p, WT or STAT1-KO A375 cells were treated with 5 ng ml⁻¹ IFNy for 24 h. RNA levels of LIMIT were quantified using RT-qPCR. All data are mean ± s.d. For o and p, n = 3 biological independent samples. Source data are available online.

**Articles**
LIMIT augments MHC-I expression

To study the function of LIMIT in tumour cells, we first knocked down LIMIT with small hairpin RNAs (shLIMIT). We used the BLAST tool to select LIMIT shRNAs that had no off-target candidates (Supplementary Tables 3–6). shLIMIT did not target GBP-coding genes (Extended Data Fig. 3a). In A375 cells, shLIMIT suppressed LIMIT expression (Fig. 2a), but had no effect on the phosphorylation of STAT1 (Fig. 2b) in response to IFNγ—suggesting that LIMIT did not affect the global IFNγ gene signalling. MHC-I and CD274 (encoding PD-L1) are IFNγ-target genes in tumour cells, shLIMIT led to a decrease in the expression of MHC-I (Fig. 2c), but not PD-L1 (Extended Data Fig. 3b), in response to IFNγ stimulation. Consistent with this human data, silencing Limit in murine melanoma cell YUMM1.7 or colon cancer cell CT26 resulted in reduced MHC-I expression in response to IFNγ (Fig. 2d–g). In A375 cells, shLIMIT affected only MHC-I expression (HLA-ABC, HLA-E and HLA-F), but also MHC-II expression (HLA-DRA and HLA-DMA), whereas LIMIT did not alter other IFNγ-signalling gene expression (Extended Data Fig. 3c). Thus, LIMIT participates in the regulation of IFNγ-induced MHC-I and MHC-II expression without altering the global IFNγ signalling pathway.

LIMIT is an interrupted gene with large introns, occupying around 17 kb in the genome. We failed to knock out the LIMIT locus using paired sgRNAs and Cas9. Given that there are five predicted STAT1/IRF1-binding sites in the LIMIT promoter, we designed four paired sgRNAs to delete these binding sites in the LIMIT promoter (Extended Data Fig. 3d). We generated A375 cells with the LIMIT-promoter deletion in all four combinations of sgRNAs. We found that IFNγ was no longer efficient at inducing the expression of LIMIT and MHC-I in tumour cells with the LIMIT promoter deletion compared with wild-type (WT) cells (Fig. 2h,i).

We also used an RNA-guided CRISPR activation system to activate LIMIT expression in tumour cells22. We established four guide RNAs targeting the promoter region of Limit (sgLimit), and co-expressed with dCas9–VPR, a tripartite transcriptional activator fused with nuclease-null Cas9, into B16 cells (Extended Data Fig. 4a). All four sgLimit guide RNAs enhanced the expression of Limit, as well as MHC-I (Extended Data Fig. 4b,c). When we transfected B16 cells with pooled sgLimit and non-targeting sgRNAs (sgNT), sgLimit induced the expression of LIMIT and MHC-I, but not PD-L1 (Fig. 2j,k). Thus, Limit selectively targets MHC-I, but not PD-L1. Together, the loss- and gain-of-function experiments demonstrate that LIMIT can alter MHC-I expression in multiple cancer cells across mice and humans by 1.5–3-fold.

We next investigated whether LIMIT-altered MHC-I expression impacts TAA-specific CD8+ T-cell-mediated tumour killing in vitro. To this end, we first genetically knocked down B2M using specific shRNAs (shB2M) in ovalbumin (OVA)-expressing B16 cells. shB2M resulted in a 1.5-fold reduction in OVA–H2Kd expression (Extended Data Fig. 4d). When B16-OVA cells carrying shFluc and shB2M were incubated with OT-I cells, we observed a decrease in OT-I-mediated shB2M-B16-OVA cell killing compared with shFluc-B16-OVA cells (Extended Data Fig. 4e–g). The data suggest that 1.5–3-fold changes in MHC-I expression controlled by LIMIT could be functionally relevant in affecting TAA-specific CTL activities. To validate this, we activated Limit in B16-OVA cells expressing shFluc and shB2M. As expected, CRISPR activation of Limit induced minimal MHC-I expression in shB2M cells compared with in control cells (Fig. 2i). Accordingly, OT-I cells mediated minimal tumour killing in shB2M-OVA-B16 cells compared with in control cells (Fig. 2m,n). The data suggest that LIMIT-induced MHC-I expression is important in TAA-specific T-cell activation and function.

LIMIT enhances anti-tumour immunity

Insufficient MHC-I expression confers tumour immune evasion and immunotherapy resistance3. To understand a role of Limit in antitumour immune responses in vivo, we inoculated control (shFluc) and Limit-silencing (shLimit) YUMM1.7 tumour cells into NOD scid γc-deficient (NSG, immune deficient) and WT C57BL/6 (immune competent) mice. Compared with control tumours, shLimit YUMM1.7 tumours grew comparably in NSG mice (Fig. 3a), whereas the tumours progressed faster in WT mice (Fig. 3b). Furthermore, we inoculated shLimit CT26 tumours into WT BALB/c mice. Again, silencing Limit resulted in enhanced CT26 tumour growth in the immune competent model (Fig. 3c). The data suggest that silencing Limit may impair anti-tumour immunity and facilitate tumour growth in an immune-dependent manner. In support of this, we detected a reduction of CD3+, IFNγ+ and TNFα+ T cells in the shLimit YUMM1.7 tumours (Fig. 3d,e). Together, silencing Limit impairs anti-tumour immunity.

To determine the expression of MHC-I and MHC-I–SIINFEKL in vivo, we established YUMM1.7 cells stably expressing OVA (YUMM1.7-OVA), and transduced them with shRNA targeting Limit or Fluc. After IFNγ treatment, we detected reduced surface expression of OVA–H2Kd in shLimit-YUMM1.7 cells (Extended Data Fig. 5a). We inoculated shLimit-YUMM1.7-OVA cells and shFluc-YUMM1.7-OVA cells into C57BL/6 mice. We next dissected tumour tissues and detected the expression of H2Dd and OVA–H2Kd in tumour cells. We observed a reduction of H2Dd and OVA–H2Kd in tumour cells. We observed a reduction of H2Dd and OVA–H2Kd in shLimit-YUMM1.7-OVA cells compared with in the control cells (Extended Data Fig. 5b–f). The data indicate that Limit may affect MHC-I and MHC-I–antigen expression in vivo.

We also inoculated control (sgNT) and Limit-activating (sgLimit) B16 cells into WT C57/BL6 mice. As expected, sgLimit (Limit activation) substantially reduced tumour growth (Fig. 3f). This was accompanied by an increase in tumour-infiltrating T-cell numbers and activation (Fig. 3g,h). B16 melanoma is a relatively insensitive tumour model to PD-L1 blockade21. Consistent with this, PD-L1 blockade failed to control sgNT B16 tumour growth in mice. Interestingly, Limit activation in B16 tumours with sgLimit sensitized the tumour response to PD-L1 blockade, as shown by a reduction in tumour progression (Fig. 3i). Together,
Fig. 2 | LIMIT augments MHC-I expression. a, A375 shFluc or shLIMIT cells were treated with IFNγ for 24 h. RNA levels of LIMIT were determined using RT-qPCR. Statistical analysis was performed using two-sided t-tests. b, A375 shFluc or shLIMIT cells were treated with IFNγ for the indicated time. Protein levels of phosphorylated STAT1 (p-Y701), STAT1 and GAPDH were determined using western blotting. One out of two experiments is shown. c, A375 shFluc or shLIMIT cells were treated with IFNγ for 48 h. Surface expression of OVA–H2Kb was determined using FACS analysis. Statistical analysis was performed using two-sided t-tests. d, B16-OVA cells carrying shFluc or shB2m were manipulated with IFNγ. RNA levels of Limit were determined 24 h after treatment. e, A375 WT cells or A375 cells with a deleted LIMIT promoter deletion were treated with IFNγ for 24 h. RNA levels of LIMIT were determined using RT-qPCR. Statistical analysis was performed using two-sided t-tests. f, A375 WT cells were transfected with dCas9–VPR, together with non-targeting sgRNA (sgNT) or sgRNA targeting the LIMIT promoter deletion were treated with IFNγ for the indicated time. Protein levels of phosphorylated STAT1 (p-Y701), STAT1 and GAPDH were determined using western blotting. One out of two experiments is shown.

Statistical analysis was performed using two-sided t-tests. All data are mean ± s.d. For a, c–i and n, n = 3 biological independent samples. Source data are available online.
LIMIT potentiates tumour immunity and sensitizes the tumour immunotherapy response.

**LIMIT cis-activates GBPs to boost MHC-I and tumour immunity**

We next examined how LIMIT affects MHC-I and tumour immunity. IncRNAs can locally regulate expression of neighbouring genes\(^\text{24}\). LIMIT is localized closely to a gene cluster, GBPs, in both human and mouse genomes (Extended Data Fig. 2a,b). We asked whether LIMIT might regulate the expression of GBPs. Silencing LIMIT reduced the levels of precursor and mature GBP mRNAs (Fig. 4a), and GBP1–5 proteins (Fig. 4b) in human A375 cells in response to IFN\(\gamma\) treatment. The data suggest that LIMIT may promote the transcription of GBPs in cis. In support of this possibility, silencing LIMIT also diminished Gbp2 expression in mouse YUMM1.7 and CT26 cells (Extended Data Fig. 6a,b). Furthermore, CRISPR activation of LIMIT induced the expression of Gbp2 in B16 cells (Fig. 4c). To test whether LIMIT could trans-regulate GBPs, we forced expression of LIMIT cDNA in A375 cells. We found that GBP1 and multiple immune factors (including IRF1, HLA-ABC, and PD-L1) were unaltered by LIMIT overexpression (Extended Data Fig. 6c). Thus, LIMIT is a cis-acting lncRNA that is capable of inducing GBP expression.
Fig. 4 | LIMIT cis-activates GBPs to boost MHC-I and tumour immunity. a, A375 shFluc or shLIMIT cells were treated with IFNγ for 24 h. RNA (a) or protein (b) levels of GBPs were determined. Statistical analysis was performed using two-sided t-tests. One out of three blots is shown. c, B16 cells were manipulated with Limit CRISPRa for 24 h. Gbp2 precursor and mature RNAs were determined. Statistical analysis was performed using two-sided t-tests. d, e, YUMM1.7 shFluc, shLimit, shGbp2 or shLimit + shGbp2 cells were treated with IFNγ. Gbp2 protein was detected 24 h after treatment (d). Surface expression of MHC-I (H2-Db) was measured 48 h after treatment (e). One out of three blots is shown. f, Statistical analysis was performed using two-sided t-tests for the tumour volume at the end point. g, h, The percentages of intratumoural CD8+ T cells or IFNγ+CD8+ T cells in YUMM1.7 tumours carrying shFluc, shLimit, shGbp2 or shLimit + shGbp2. n = 5 animals. Statistical analysis was performed using two-sided t-tests. h, MC38 shFluc or shGbp2 cells were treated with IFNγ. Surface staining of H2-Db was determined 48 h after treatment. Statistical analysis was performed using two-sided t-tests. i, j, Tumour growth curves of MC38 shFluc and MC38 shGbp2 cells. Tumour-bearing mice were treated with IgG or anti-PD-L1 antibodies. n = 6 (shFluc) or n = 7 (shGbp2) animals. Statistical analysis was performed using two-sided t-tests for the tumour volume at the end point. j, A375 cells were treated with the indicated cytokines for 24 h. Protein levels of GBP1-5 were determined. One out of two experiments is shown. k–m, A375 WT or Gbp1-5 KO cells were treated with IFNγ. GBP1-5 protein (k) and MHC-I-related gene transcripts (l) were determined 24 h after treatment. MHC-I surface expression (m) was determined 48 h after treatment. One out of three blots is shown. Statistical analysis was performed using two-sided t-tests. All data are mean ± s.d. For a, c, e, h, i and m, n = 3 biological independent samples. Source data are available online.
Among Gbp family members, Gbp2 is a predominant Gbp family member in mouse cells (Extended Data Fig. 6d). To test whether LIMIT may regulate MHC-I through Gbps, we established stable YUMM1.7 cells carrying shFluc, shLimit, shGbp2 or shLimit+shGbp2. We found that, in response to IFNγ stimulation, shLimit and shGbp2 led to a comparable decrease in Gbp2 and MHC-I expression; simultaneously silencing LIMIT and Gbp2 failed to additionally alter Gbp2 and MHC-I expression (Fig. 4d,e). Moreover, we wondered whether GBP overexpression may rescue downregulated MHC-I expression in Limit-knockdown tumour cells. We forced expression of GBP1 in shLIMIT A375 cells (GBP1OE) and treated these cells with IFNγ. We observed that shLIMIT resulted in reduced MHC-I expression in control cells, but not in Gbp1OE cells (Extended Data Fig. 6e). Expression of PD-L1 and IRF1 was not affected by shLIMIT or GBP1OE (Extended Data Fig. 6e,f). Thus, LIMIT may regulate MHC-I expression in a GBP-dependent manner. We next inoculated YUMM1.7 cells with Limit and/or Gbp2 silencing in C57BL/6 mice. Silencing LIMIT and silencing Gbps similarly resulted in faster tumour growth compared with the control group, whereas simultaneously silencing LIMIT and Gbps did not further affect tumour progression (Fig. 4f). Furthermore, we detected a decrease in tumour-infiltrating T-cell numbers and activation in shLIMIT tumours, shGbp2 tumours and shLimit+shGbp2 tumours (Fig. 4g and Extended Data Fig. 6g). Together, LIMIT augments MHC-I expression and tumour immunity in a GBP-dependent manner.

Gbps are IFNγ-responsive genes in fibroblasts and macrophages in the context of host defence against pathogens. However, a role of Gbps in cancer immunity is unknown. Given that silencing Gbps reduced MHC-I expression and CD8+ T-cell activation (Fig. 4g and Extended Data Fig. 6g), we hypothesized that Gbps might affect the efficacy of cancer immunotherapy. To test this hypothesis, we silenced Gbp2 in MC38 cells, a tumour model that is sensitive to immunotherapy. As expected, silencing Gbp2 in MC38 cells reduced MHC-I expression after IFNγ treatment (Fig. 4h), and largely abrogated the efficacy of PD-L1 blockade (Fig. 4i). This, along with the aforementioned data, suggest that LIMIT and Gbps are involved in controlling cancer immunotherapy efficacy. In support of this possibility, clinical data analysis revealed that high levels of GBP expression are correlated with LIMIT and MHC-I expression and immunotherapy response (Extended Data Fig. 6h–i) in patients with melanoma. Furthermore, levels of GBP expression were positively associated with patient survival (Extended Data Fig. 6k). To validate whether Gbps are IFNγ-responsive genes in cancer cells, we stimulated A375 cells with IFNγ and other cytokines. Gbps were induced by IFNγ, but minimally affected by other immune cytokines (Fig. 4j). We next used the CRISPR–Cas9 system to target the shared sequences among GBP1–5, and generated GBP1–5-KO A375 cells (Fig. 4k). We observed that IFNγ poorly stimulated MHC-I gene machinery transcripts (Fig. 4l) and surface HLA-ABC proteins in GBP KO A375 cells (Fig. 4m). Thus, LIMIT cis-activates Gbps to boost MHC-I machinery and tumour immunity.

Gbps activate HSF1 to stimulate MHC-I and tumour immunity

To demonstrate how Gbps may regulate MHC-I expression and tumour immunity, we forced expression of Gbps in A375 cells. Interestingly, overexpression of Gbps increased human MHC-I expression as shown by quantitative PCR with reverse transcription (RT–qPCR; Fig. 5a), membrane surface staining (Fig. 5b) and western blotting (Fig. 5c). The data suggest that Gbps may activate MHC-I at the transcriptional level. Consistently, overexpression of Gbp2 increased the expression of mouse MHC-I in YUMM1.7 and B16 cells (Fig. 5d).

To identify the transcription factor(s) that regulate MHC-I through Gbps in response to IFNγ, we performed bioinformatics prediction using PROMO2. We found that eight transcription factors were altered by IFNγ in A375 cells that may target HLA-ABC, HSPA5, CALR and TAP1. Besides several well-known factors, HSF1 activity was highly induced by IFNγ (Extended Data Fig. 7a). By processing chromatin immunoprecipitation coupled with sequencing (ChIP–seq) datasets in ENCODE, we found that both STAT1 and HSF1 were enriched at the promoters of MHC-I-associated genes with different binding patterns (Extended Data Fig. 7b). We performed a ChIP assay using anti-HSF1 antibodies in IFNγ-stimulated A375 cells. HSF1 was enriched at the promoters of HLA-ABC, HSPA5, CALR and TAP1, but not HPRT1, a negative control (Fig. 5e). The results suggest that HSF1 is a transcription factor for MHC-I. HSF1 is usually activated by proteostasis interruption.

To test whether activation of HSF1 enhances MHC-I expression, we treated A375 cells with a list of stressors: heat shock, oxidative stress, inhibitors of translation (puromycin), proteasome (MG-132) and chaperone (17-AAG). Interestingly, these stressors universally stimulated MHC-I expression, whereas KRIBB11, an HSF1 inhibitor, reduced this effect (Fig. 5f and Extended Data Fig. 7c). Thus, activation of HSF1 generally induces MHC-I expression.

We next questioned whether Gbps could activate HSF1. Forced expression of Gbps induced the luciferase activity of HSF1 reporter HSE–Luc (Fig. 5g), as well as the phosphorylation of HSF1 (Fig. 5h). This suggests that Gbps could activate HSF1. IFNγ failed to induce HSPA5 expression in GBP-KO A375 cells (Fig. 5i). Thus, IFNγ activates HSF1 by inducing GBP expression. Furthermore, treatment with KRIBB11, an HSF1 inhibitor, abrogated the upregulation of MHC-I mediated by GBP1 overexpression (Fig. 5j). Thus, Gbps stimulate MHC-I expression in an HSF1-dependent manner.

To solidify the mechanistic relationship between Gbps and HSF1, we silenced Gbp2 and/or Hsf1 in MC38 cells (Fig. 5k). After IFNγ treatment, silencing of Gbp2 or Hsf1 alone diminished MHC-I expression, but simultaneously silencing Gbp2 and Hsf1 failed to additionally modulate MHC-I expression (Fig. 5l). To demonstrate the functional relevance of the interplay between Gbps and HSF1 in tumour immunity, we inoculated MC38 tumour cells expressing shFluc, shGbp2, shHsf1 or shGbp2+shHsf1 into C57BL/6 mice. In comparison to shFluc controls, silencing Gbp2 and silencing Hsf1 comparably accelerated tumour growth (Fig. 5m) and diminished tumour-infiltrating T-cell numbers and activation (Fig. 5n and Extended Data Fig. 7d). Moreover, simultaneously silencing Gbp2 and Hsf1 failed to further affect tumour growth and tumour-infiltrating T cells (Fig. 5m,n and Extended Data Fig. 7d). Together, Gbps stimulate MHC-I expression and antitumour immunity by activating HSF1.

The LIMIT–GBP–HSF1 axis drives MHC-I and tumour immunity

We next examined how Gbps activate HSF1 to alter MHC-I expression and tumour immunity. Under normal conditions, monomeric HSF1 is associated with and suppressed by chaperones, such as HSP90 (ref. 34). Interruption of their interaction permits trimerization and accumulation of HSF1 in the nucleus, resulting in transcriptional activation of its target genes. We hypothesized that Gbps may disturb the association between HSP90 and HSF1, resulting in HSF1 activation. To test this possibility, we treated A375 cells with IFNγ and performed co-IP using anti-HSP90 antibodies. We found that IFNγ-induced endogenous Gbps were associated with HSP90 (Fig. 6a). Furthermore, we transfected A375 cells with exogenous Flag–GBP1 and performed the co-IP experiment with Flag-antibody. HSP90 was detected in the IP product of Flag–GBP1-transfected cells, but not vector-transfected control cells (Fig. 6b). Immunofluorescence staining demonstrated that Gbps and HSP90 were largely colocalized in the cytoplasm (Fig. 6c). When we transfected HEK293T cells with increasing doses of GBP1 plasmids, HSP90-associated HSF1 was reduced in a dose-dependent manner.
Fig. 5 | GBPs activate HSF1 to stimulate MHC-I expression and tumour immunity. a–c, A375 cells were forced to express GBPs. MHC-I RNA (a), surface expression (b) or total protein (c) levels were determined 24 h (a) or 48 h (b,c) afterwards. Statistical analysis was performed using two-sided t-tests. One out of two blots is shown. d, MHC-I surface expression in YUMM1.7 or B16 cells after Gbp2 overexpression. Statistical analysis was performed using two-sided t-tests. e, HSF1 chromatin IP for the indicated gene promoters was performed in IFNγ-pretreated A375 cells. Statistical analysis was performed using two-sided t-tests. f, A375 cells were treated with 17-AAG to activate HSF1. Protein levels of HLA-ABC were determined 48 h after treatment. One out of two experiments is shown. g, HSF1 was induced in A375 cells and treated with KRIBB11. MHC-I surface expression was determined 48 h afterwards. Statistical analysis was performed using two-sided t-tests. h, A375 cells were forced to express GBP1. The levels of the indicated proteins were determined 12 h afterwards. One out of two experiments is shown. i, A375 cells were forced to express GBPs. MHC-I RNA (i), surface expression (j) or 48 h (k) afterwards. Statistical analysis was performed using two-sided t-tests. k, MC38 shFluc, shGbp2, shHsf1 or shGbp2 + shHsf1 cells were treated with IFNγ. The levels of the indicated proteins (k) or MHC-I surface expression (l) were determined 48 h afterwards. One out of two experiments is shown. Statistical analysis was performed using two-sided t-tests. l, Tumour growth curves of MC38 shFluc, shGbp2, shHsf1 and shGbp2 + shHsf1 cells in C57BL/6 mice. n = 5 animals. Statistical analysis was performed using two-sided t-tests for the tumour volume at the end point. m, Percentages of intratumoural IFNγ+CD8+ T cells or TNFα+CD8+ T cells in MC38 tumours carrying shFluc, shGbp2, shHsf1 or shGbp2 + shHsf1, n = 5 biological independent samples. Statistical analysis was performed using two-sided t-tests. All data are mean ± s.d. For a, b, d, e, g, j and l, n = 3 biological independent samples. Source data are available online.
Fig. 6 | The LIMIT–GBP–HSF1 axis drives MHC-I and tumour immunity. a, Co-IP analysis of GBP1–5 using anti-HSP90 antibodies in IFNγ-pretreated A375 cells. One out of three experiments is shown. WB, western blot. b, Co-IP analysis of HSP90 using anti-Flag antibodies in A375 cells overexpressing Flag–GBP1. The asterisk indicates the band shift of HSP90 after GBP1 overexpression. One out of two experiments is shown. c, Immunofluorescence staining of GBP1 and HSP90 in IFNγ-pretreated A375 cells. One out of four images is shown. d, HEK293T cells were forced to express Flag–HSF1 and indicated amount (µg) of Flag–GBP1. Co-IP analysis of HSF1 or GBP1 with anti-HSP90 antibodies was performed 24 h afterwards. One out of two experiments is shown. e, A375 cells were treated with HSP90 inhibitor (HSP90i), or forced to express GBP1. The indicated proteins were detected in A375 cells. One out of three experiments is shown. WB, western blot.

f, i, j, Protein (h) or surface expression (i) levels of the indicated genes were determined 48 h afterwards. Statistical analysis was performed using two-sided t-tests. h, YUMM1.7 shFluc or shHSf1 cells were treated with IFNγ. Total protein (h) or surface staining (i) levels of the indicated genes were determined 48 h afterwards. One out of two experiments is shown. Statistical analysis was performed using two-sided t-tests. j, k, YUMM1.7 (NSG) or (WT) cells were transplanted to NSG mice and tumour growth curves of YUMM1.7 shFluc or shHSf1 tumours were measured. n = 5 biological independent samples. Statistical analysis was performed using two-sided t-tests. m, A375 shFluc or shLIMIT cells were transfected with HSE-LUC and PRL-5V40 overnight, and then treated with IFNγ for a further 48 h. HSF1 transcriptional activity is depicted as the relative luciferase activity. Statistical analysis was performed using two-sided t-tests. n, B16 cells were manipulated with LIMIT CRISPRa and treated with KRI/B11. Surface expression of MHC-I (H2-D^b) was determined 48 h afterwards. Statistical analysis was performed using two-sided t-tests. All data are mean ± s.d. For f, g, i, m and n, n = 3 biological independent samples. Source data are available online.
manner (Fig. 6d). The data suggest that GBPs interacted with HSP90 and this interaction disrupted the association between HSF1 and HSP90. HSP90 is a chaperone for multiple protein folding and stability, we questioned whether GBPs may alter the chaperone activity of HSP90. Although HSP90 inhibitor suppressed the expression of HSP90 client proteins (such as RANF1, BCL2 and CDK4)\textsuperscript{48}, overexpression of GBPs failed to do so (Fig. 6e). Thus, GBPs interact with HSP90, and release HSP90-associated HSF1, but do not alter HSP90 activity.

We next directly examined the role of HSF1 in MHC-I expression. We treated A375 cells with IFNγ in the presence of the HSF1 inhibitor KRIBB11. As expected, treatment with KRIBB11 reduced IFNγ-stimulated mRNA expression of MHC-I-related genes, including HLA-ABC, TAP1, HSPA5 and CALR, but not IFRF1 (Fig. 6f). Interestingly, KRIBB11 reduced IFNγ-induced MHC-I expression, but had a minimal effect on PD-L1 expression (Fig. 6g).

Thus, HSF1 can regulate IFNγ-induced MHC-I expression without altering the global IFNγ signalling.

To investigate whether HSF1-regulated MHC-I was functional, we cultured B16-OVA with OT-1 cells in the presence of KRIBB11 and IFNγ. KRIBB11 inhibited IFNγ-induced expression of OVA-bound MHC-I (Extended Data Fig. 8a). Consistent with this, KRIBB11 also suppressed OT-1-cell-mediated cytotoxic effects on B16-OVA (Extended Data Fig. 8b). To extend our observations to additional tumours, we silenced Hsf1 with shHsf1 in YUMM1.7 and CT26 cells. Silencing Hsf1 resulted in a decrease in MHC-I expression in YUMM1.7 (Fig. 6h,i) and CT26 cells (Extended Data Fig. 8c) in response to IFNγ stimulation. In YUMM1.7 cells, silencing Hsf1 failed to affect Gbp2 expression in response to IFNγ (Fig. 6h), indicating that Gbp2 is not an HSF1-target gene. In shHsf1 YUMM1.7 cells, KRIBB11 failed to suppress IFNγ-stimulated MHC-I expression (Extended Data Fig. 8d). The data suggest that HSF1 enhanced MHC-I expression in response to IFNγ, and Hsf1 is the mechanistic target of KRIBB11 to regulate MHC-I.

Given that HSF1 affected MHC-I expression, we hypothesized that HSF1 regulated anti-tumour immunity in vivo. To test this hypothesis, we inoculated control and shHsf1 YUMM1.7 tumour cells into NSG and C57BL/6 mice. We observed that silencing Hsf1 partially slowed down YUMM1.7 tumour progression in NSG mice (Fig. 6j), supporting that Hsf1 helped to maintain protein homeostasis and tumour progression in the immune-deficient model. However, silencing Hsf1 substantially accelerated YUMM1.7 tumour growth in WT C57BL/6 mice (Fig. 6k). The data indicate that Hsf1 may surprisingly promote potent anti-tumour immunity in the immune competent model. In support of this, we detected a decrease in the percentages of tumour-infiltrating CD3+, Ki67+, IFNγ+ and TNFα+ T cells (Fig. 6l) and Extended Data Fig. 8e) in the shHsf1 YUMM1.7 tumours compared with the shFluc scramble controls. Furthermore, we inoculated shHsf1 CT26 cells into WT BALB/c mice. Again, silencing Hsf1 resulted in enhanced CT26 tumour growth (Extended Data Fig. 8f). This was accompanied by a decrease in the percentages of tumour-infiltrating CD3+, Ki67+, IFNγ+ and TNFα+ T cells (Extended Data Fig. 8g,h). Together, the data suggest that the GBP–HSF1 axis drives MHC-I expression and antitumour immunity.

To mechanistically connect HSF1 and LIMIT, we silenced LIMIT in A375 cells. Silencing LIMIT reduced the transcriptional activity of HSF1 in response to IFNγ, as determined by luciferase reporter assay (HSE-LUC) (Fig. 6m). The data suggest that LIMIT contributes to HSF1 activation in response to IFNγ. To test a potential involvement of HSF1 in the LIMIT-mediated induction of MHC-I, we stimulated LIMIT through CRISPR activation in B16 cells in the presence of KRIBB11. We observed that MHC-I upregulation, induced by LIMIT-activation, was abrogated by a HSF1 inhibitor (Fig. 6n). The data suggest that LIMIT boosts MHC-I expression in an HSF1-dependent manner.

Finally, we analysed a link between LIMIT, GBPs and HSF1 in the context of MHC-I expression, tumour immunity and immunotherapy in patients with cancer. Clinical analysis showed that HSF1 signalling genes were correlated with MHC-I expression, CD8+ T-cell infiltration and patient survival (Extended Data Fig. 9a–c). In an immune checkpoint blockade study in patients with basal cell carcinoma\textsuperscript{41}, single-cell RNA-seq analysis revealed two tumour clusters; one tumour cluster was more sensitive to anti-PD-1 treatment as shown by a largely reduced tumour population (Extended Data Fig. 9d). Interestingly, this immune-checkpoint-sensitive tumour cluster expressed higher levels of HSF1-signalling genes as well as MHC-I gene machinery (Extended Data Fig. 9e). Moreover, in an immune checkpoint blockade study in patients with melanoma\textsuperscript{42}, proteomic analysis demonstrated that the protein expression of GBPs, HSF1 signalling genes and MHC-I were higher in clinical responders compared with those in non-responders (Extended Data Fig. 9f). Moreover, we observed a positive correlation between GBP1 and HSF1 signalling genes in human cancers (Extended Data Fig. 9g). The data support that the LIMIT–GBP–HSF1 axis may activate MHC-I expression and favour anti-tumour immunity (Extended Data Fig. 10).

**Discussion**

Humans have 30,000–60,000 lncRNAs. However, the identities and biological functions of the vast majority of these potential lncRNAs remain poorly understood. In the cancer biology field, lncRNAs have been largely studied in the immune deficient model, leaving a knowledge gap of lncRNAs in the context of the immune system. A handful of lncRNAs are reported to affect immune cell function\textsuperscript{43,44}, cancer progression and chemotherapy efficacy\textsuperscript{45,46}. However, whether specific lncRNAs are involved in antitumour immunity and immunotherapy response remains unanswered. Here we identified that LIMIT is an IFNγ-responsive lncRNA in both human and mouse cells. LIMIT can induce MHC-I and MHC-II expression, promoting T-cell-mediated tumour immune response and enhancing immunotherapy efficacy. Thus, LIMIT is a tumour immunogenic lncRNA.

The IFNγ signalling pathway has a key role in determining therapeutic response to cancer immunotherapy\textsuperscript{47} by multiple mechanisms\textsuperscript{48,49,50}. Genetic mutations in IFNγ signalling genes contribute to checkpoint blockade resistance in patients with cancer\textsuperscript{51–54}. However, IFNγ signalling can induce inhibitory PD-L1 expression\textsuperscript{55,56}. Thus, it is ideal to identify and target a key IFNγ signalling gene that selectively mediates anti-tumour immunity, rather than tumour immune evasion. In line with this notion, we demonstrate that LIMIT mediates MHC-I and MHC-II upregulation, but has no effect on PD-L1 expression in response to IFNγ. Thus, LIMIT may be uniquely positioned to be an immunogenic target for cancer immunotherapy.

Several strategies have been proposed to therapeutically target pathogenic lncRNAs\textsuperscript{57}. However, how to elevate the levels of beneficial lncRNAs remains challenging. As cis-acting lncRNAs function locally, forced expression of these lncRNAs may be incapable of locating precisely\textsuperscript{58}. Although trans-acting lncRNAs may function through specific secondary structures, overexpression of these lncRNAs may not be able to generate their natural structures due to missing appropriate RNA chaperones\textsuperscript{59}. Using a RNA-guided CRISPR activation strategy\textsuperscript{43}, we directly activated LIMIT expression in tumour cells in preclinical models. RNA-guided CRISPR LIMIT activation can drive tumour MHC-I expression and potentiate checkpoint blockade therapy. Given that a loss of MHC-I and IFNγ gene signatures frequently occurs in human tumours, we suggest that CRISPR activation of beneficial lncRNAs, such as LIMIT, can rescue tumour MHC-I expression and be a potential therapeutic approach.

While searching for the mechanism by which LIMIT affects tumour immunity, we elucidated that LIMIT targets GBPs in an
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Luciferase activity assay. A375 cells were transfected with HSE-LUC and PRL-SV40 (Addgene, 27163) for 24 h, together with PCL-neo (vector) or GBP1 or GBP2 for 48 h. A375 shFluc or A375 shLIMIT cells were transfected with HSE-LUC and PRL-SV40 (Addgene, 27163) for 24 h, and then treated with IFNγ for an additional 48 h. Luciferase activity for firefly luciferase (HSE-LUC) and Renilla luciferase (PRL-SV40) were measured using the Dual-Luciferase Reporter Assay System (Promega). Relative firefly luciferase activity was normalized to Renilla luciferase activity.

Surface staining and flow cytometry analysis (FACS). Cells were trypan blue and washed with MABS buffer (PBS, 2% FBS, 1 mM EDTA). Surface staining was performed by adding the following antibodies to the cell suspension in 50 μL MABS buffer: anti-HLA-ABC (G46-2.6, BD Biosciences), anti-H2-D^d (KH95, BD Biosciences), anti-H2-D^Q (34-1-12, BD Biosciences), anti-OVA-H2-K^b (Biocytin-D1.21, eBioscience) and anti-PD-L1 (MH1, BD Biosciences) antibodies. After incubating for 30 min, cells were washed with MABS buffer and analysed using the BD Fortessa flow cytometer.

qPCR analysis. Total RNA was isolated from cells by column purification (Direct-zol RNA Miniprep Kit, Zymo Research). cDNA was synthesized using the ReverTaid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) with random hexamer primers. qPCR was performed on CDNA using the Fast SYBR Green Master Mix (Thermo Fisher Scientific) on a StepOnePlus Real-Time PCR System (Thermo Fisher Scientific). Gene expression was quantified using the primer in the Supplementary Table. Ct values were calculated using the ΔΔCt method and fold change was expressed as fold change by normalizing to the controls.

Northern blotting. Northern blot analysis was performed with 10 μg of total RNAs prepared from IFNγ-, IFNγ- and TNFα-pretreated A375 cells. RNAs were resolved by electrophoresis agarose gel electrophoresis in (Ambion) and transferred to Hybond-XL membranes (GE Healthcare). LIMIT was detected using digoxin-labelled DNA probes with the DIG Northern Starter Kit (Roche). A list of the probes sequences targeting LIMIT is provided in Supplementary Table 7.

RACE. RACE was performed to identify the cDNA ends of human LIMIT or mouse LIMIT using the SMARTer RACE cDNA Amplification Kit (Clontech). A list of the primers for RACE is provided in Supplementary Table 8.

Clone of full-length LIMIT. After obtaining the cDNA end sequences, full-length LIMIT was PCR amplified and inserted into PCL-neo plasmid between Xhol and NotI. A list of the cloning primers for human LIMIT and mouse LIMIT is provided in Supplementary Table 8.

Cell fractionation for RT–PCR. IFNγ-pretreated A375 cells were collected from 15 cm plates by scraping and were washed once with cold PBS. Cells were pelleted by centrifugation at 700g for 5 min and lysed with hypotonic lysis buffer (10 mM Tris (pH 7.5), 10 μM NaCl, 3 mM MgCl2, 0.3% (v/v) NP-40, 10% (v/v) glycerol) to collect the cytoplasmatic fraction. Cytoplasmatic RNA was obtained by ethanol precipitation overnight at −20 °C, followed by re-extraction using TRIZOL reagent. The remaining nuclear pellet was washed three times with the hypotonic lysis buffer, followed by extraction with TRIZOL reagent. The nuclear RNA was used as controls for nuclear or cytoplasmic RNA, respectively. A list of the primers for RT–PCR was used for the indicated primers. Unspliced and mature ACTB was used as controls for nuclear or cytoplasmic RNA, respectively. A list of the primers for ACTB is provided in Supplementary Table 8.

Western blot analysis. Cells were washed in cold PBS and lysed in 1x RIPA lysis buffer (Pierce) with 1x protease inhibitor (Pierce). Lysates were incubated on ice for 10 min and cleared by centrifugation at 15,000g for 15 min. Protein concentration was quantified using a BCA protein assay kit (Thermo Fisher Scientific). Protein (30 μg) was mixed with sample buffer (Thermo Fisher Scientific) with β-ME and denatured at 95 °C for 5 min. The samples were separated by SDS-PAGE and transferred to a nitrocellulose membrane (Bio-Rad). Membranes were blocked with 5% w/v non-fat dry milk and incubated with primary antibodies overnight at 4 °C and HRP-conjugated secondary antibodies (CST) for 1 h at room temperature. Signal was detected using Clarity and Clarity Max Western ECL Blotting Substrates (Bio-Rad) and captured using the ChemiDoc Imaging System (Bio-Rad).

Data analysis. All experiments were performed in triplicate, and error bars indicate standard deviations. See Supplementary Table for the details of the reagents and antibodies used in this study.
**Co-IP analysis.** Cells were collected with IP lysis buffer (Pierce, 87787) plus protease inhibitor. Protein concentration was determined using the BCA protein assay kit. Protein samples (200–500 μg) were added to 1–3 μg primary antibodies (anti-HSP90) (Proteintech, 13171) or anti-HSF1 (CST, 12972), and incubated with gentle rocking at 4 °C overnight. Samples were then further incubated with 20 μl Protein A/G PLUS-Agarose (Santa Cruz, sc-2003) for 2 h at 4 °C, and centrifuged at 7,500 rpm for 30 s at 4 °C. Cell pellets were washed four times with IP lysis buffer, resuspended with 40 μl 2x sample buffer with β-ME, and heated for 5 min at 95 °C. The denatured protein samples were analysed using western blotting. For Flag IP, cell lysates were incubated with 20 μg EZview Red ANTI-FLAG M2 Affinity Gel (Sigma-Aldrich), and washed, denatured and analysed as described above.

**Immunofluorescence staining.** A375 cells mounted onto coverslips were treated with IFNγ for 24 h. After washing twice with PBS, cells were fixed with 4% PFA for 15 min and washed twice with PBS for 5 min each. The cells were then permeabilized with 0.5% Triton X-100 in PBS for 10 min and rinsed twice with PBS for 5 min each. Antigens were blocked with 10% normal goat serum in PBS for 30 min. Primary antibodies were then added at 1:50 dilutions of mouse anti-human HSPB1-5 antibodies (Santa Cruz, 166960) or rabbit anti-human HSPA90 antibodies (CST, 48773), and incubated at 4 °C overnight. The cells were then washed and incubated with 1:500 dilutions of Qdot 650-labelled secondary goat anti-mouse antibodies (Thermo Fisher Scientific, Q11002) or AF488-labelled secondary goat anti-rabbit antibodies (Thermo Fisher Scientific, A11034), and then mounted onto glass slides using ProLong Gold reagent containing DAPI. Confocal fluorescence images were collected using a ×63 oil-immersion objective (Leica SP5 Inverted 2-Photon FLIM confocal).

**ChIP analysis.** ChIPs were performed using cross-linked chromatin from IFNγ-treated A375 cells and either anti-HSF1 antibodies (CST, 12972) or normal rabbit IgG (CST, 2729), using the Simple ChIP Plus Enzymatic Chromatin IP Kit (Magnetic Beads) (CST, 9005). The enriched DNA was quantified by qPCR using the primers listed in Supplementary Table 8. The amount of immunoprecipitated DNA in each sample is represented as the signal relative to total amount of input chromatin, which is equivalent to 1.

**OT-I cell isolation and coculture with OVA+ tumour cells.** C57BL/6-Tg(TcraTcrb)1100Mjb/J (OT-I) mice (JAX stock, 003831) were purchased from the Jackson Laboratory. The spleen was homogenized, and single cells were suspended in 2 ml red blood cell lysis buffer (Sigma-Aldrich) for 1 min. The splenocytes were pelleted, washed and resuspended at 2×10⁶ cells per ml in RPMI culture medium containing 1 μg/ml OVA257-264 peptide, 5 μg/ml mouse recombinant IL-2 and 40 μM 2-mercaptoethanol. The cells were incubated at 37 °C for 5 d. To set up the coculture of OT-I and OVA+ tumour cells, splenocytes were collected after activation for 5 d. OT-I cells were purified using the EasySep mouse CD8+ T Cell Isolation Kit (Stemcell). B16-OVA cells were seeded overnight. OT-I cells were then added into the culture at different ratios. All cells were collected by trypsinization and analysed using flow cytometry (FACS).

**Bone-marrow-derived dendritic cells and macrophages.** Bone marrow was isolated from C57BL/6 mouse femurs and cultured in RPMI 1640 complete medium with 20 nmol/l GM-CSF (R&D). Cells were incubated at 37 °C under 5% CO₂. An additional 10 ml medium with 20 ng/ml GM-CSF was added at day 3. On day 7, non-adherent and loosely adherent cells in the culture supernatant were collected by gentle washing with PBS, and considered to be bone-marrow-derived dendritic cells. The adherent cells were considered to be bone-marrow-derived macrophages.

**Intratumoural immune cell profiling.** To quantify intratumoural T cells and T-cell effector cytokine expression, single-cell suspensions were prepared from fresh tumour tissues by physically passing them through 100 μm cell strainers. Immune cells were enriched by density gradient centrifugation. For cytokine staining, intratumoural immune cells were incubated in RPMI culture medium containing PMA (5 ng/ml), ionomycin (500 nmol/l), brefeldin A (1:10,000) and monensin (1:1,000) at 37 °C for 4 h. Two to three microlitres of anti-CD45 (30-F11, BD Biosciences), anti-CD8 (30-F11, BD Biosciences), anti-CD3 (145-2C11, BD Biosciences), anti-CD4 (RM4-5, BD Biosciences) and anti-CD8 (53-6.7, BD Biosciences) antibodies were added for 20 min for surface staining. The cells were then washed and resuspended in 1 ml of freshly prepared Fix/Perm solution (BD Biosciences) at 4 °C overnight. After being washed with Perm/Wash buffer (BD Biosciences), the cells were stained with 2–3 μl anti-Ki67 (B56, BD Biosciences), anti-TNF (MP6-XT22, BD Biosciences) and anti-IFNγ (XM61.2, BD Biosciences) antibodies for 30 min, washed and fixed in 4% formaldehyde (Sigma-Aldrich). All of the samples were read using the LSR Fortessa cytometer and analysed using FACS DIVA v8.0 (BD Biosciences).

**Signature score computation.** We used normalized expression of genes to define the following signatures: CD8+ T-cell infiltration (CD4A, CD4B, PRF1 and GZMIB), MHC-I expression (HLA-A, HLA-B and HLA-C) and HSF1 signalling (HSPIA1, HSPIA1B, HSPIA5 and HSPI90B1).

**Statistical analysis.** For cell-based experiments, biological triplicates were performed in each single experiment in general, unless otherwise stated. For animal studies, no less than five replicates per group were performed. Animals were randomized into different groups after tumour cell inoculation. The investigators were not blinded to allocation during experiments and outcome assessment. Data are shown as mean values ± s.d. Statistical analysis was performed using GraphPad Prism 8 (GraphPad Software), following the manuals of GraphPad Prism 8.0 and an online resource (http://xena.ucsc.edu/). Two-tailed two-sided t-tests were used to compare treatment groups with control groups; survival function was estimated using the Kaplan–Meier methods and log-rank tests were used to calculate statistical differences.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

The RNA-seq data (GSE99299) and processed single-cell data (GSE123814) were obtained from Gene Expression Omnibus (GEO). The MS proteomics data (PXD006003) were obtained from PRIDE repository. The TCGA cancer datasets were obtained from UCSC Xena (http://xena.ucsc.edu/). The RNA-seq data and clinical information for immune checkpoint blockade clinical trials were provided by the respective corresponding authors. All raw data supporting the findings of this study are available from the corresponding author on request. Source data are provided with this paper.

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**Author contributions**

G.L. and W.Z. conceived the idea, designed the experiments and composed the paper. G.L. conducted experiments. I.K. assisted in FACS analysis. J.N., S.W, S.G. and L.V. assisted in animal experiments. X.L., S.L. and J.L. assisted in bioinformatics analysis. J.Z., G.L. and W.Z. conceived the idea, designed the experiments and composed the paper.

**Competing interests**

W.Z. has served as a scientific advisor or consultant for NGM, Cstone, Oncopia and Hengenix. All of the other authors declare no competing interests.

**Additional information**

Extended data is available for this paper at https://doi.org/10.1038/s41556-021-00672-3. Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41556-021-00672-3. Correspondence and requests for materials should be addressed to W.Z. Peer review information Nature Cell Biology thanks Wenyi Peng and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available. Reprints and permissions information is available at www.nature.com/reprints.
Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | LIMIT correlates to effector immune genes across multiple cancer types. a-l, Correlation of LIMIT with IFNG, MHC-I, or CD8 in human patients with sarcoma (SARC) (a-c), colon cancer (COAD) (d-f), breast cancer (BRCA) (g-i), and kidney cancer (KIRC) (j-l). P value by 2 sided linear regression. Source data are provided.
Extended Data Fig. 2 | Genetic loci and sequences of human LIMIT and mouse Limit. a-b, Genetic locus and genomic sequence of human LIMIT (a) or mouse Limit (b). c, Blast alignment of human LIMIT with GBP1P1 or GBP1.
Extended Data Fig. 3 | LIMIT augments MHC-I expression. **a**, Schematic diagram showing the alignment among LIMIT, GBP, and shLIMIT. The shLIMIT target sequences are not present in GBP coding genes. **b**, A375 shFluc, shLIMIT a, and shLIMIT b cells were treated with IFNγ for 48 hours. Surface expression of PD-L1 was determined by flow cytometry (FACS). mean ± SD, n = 3 biological independent samples, P value by 2-sided t-test. **c**, A375 shFluc and shLIMIT cells were treated with IFNγ for 24 hours. RNA levels of indicated genes were determined. mean ± SD, n = 3 biological independent samples, P value by 2-sided t-test. **d**, Schematic diagram of the LIMIT promoter. The locations of 5 STAT1/IRF1-binding motifs and 4 sgRNAs capable of deleting the STAT1/IRF1 binding sites are indicated. Source data are provided.
Extended Data Fig. 4 | LIMIT augments MHC-I expression. a, Schematic diagram of CRISPR activation targeting Limit. The transcriptional activator VPR was directed to the promoter of Limit by the interaction between guide RNAs and dCas9. b–c, B16 cells were transfected with dCas9-VPR alone or together with 4 sgRNAs. Subsequently, RNA levels of Limit (b) and surface expression of MHC-I (H2-Db) (c) were detected 24 and 48 hours post transfection, respectively. mean ± SD, n = 4 biological independent samples, P value by 2-sided t-test. d, B16-OVA cells stably expressing shFluc, shB2m a, and shB2m b were treated with IFNγ for 48 hours. Surface expression of OVA-H2Kb was determined by FACS. mean ± SD, n = 3 biological independent samples, P value by 2-sided t-test. e, B16-OVA cells were co-cultured with OT-I cell for 48 hours. Tumor cell death was determined by PI staining. f–g, B16-OVA cells carrying shFluc or shB2m were co-cultured with OT-I cells at a 1:4 ratio. Dot plots show the CD45− tumor cells. Tumor cell death was determined by PI in CD45− tumor cells. Dot plots (f) and statistical results (g) are shown. mean ± SD, n = 3 biological independent samples, P value by 2-sided t-test. h, Bone marrow derived dendritic cells (BMDC) (h) or macrophages (BMDM) (i) were treated with IFNγ for 24 hours. RNA levels of Limit were determined by qRT-PCR. mean ± SD, n = 3 biological independent samples, P value by 2-sided t-test. j, BMDM were transfected with 5’FAM-labbled siRNA targeting Fluc or Limit. Dot plots show FSC vs. SSC and FITC vs. SSC gating. The FITC gating indicates the cells with positive siRNA transfection. k, BMDM were transfected with 5’FAM-labbled siRNA targeting Fluc or Limit, and treated with IFNγ for 48 hours. Surface expression of MHC-I (H2-Db) were determined by FACS. mean ± SD, n = 3 biological independent samples, P value by 2-sided t-test. Source data are provided.
Extended Data Fig. 5 | LIMIT augments antigen-loaded MHC-I expression in vivo. a, YUMM1.7-OVA cells carrying shFluc or shLimit were treated with IFNγ for 48 hours. Surface expression of OVA-H2Kb were determined by FACS. mean ± SD, n = 5 biological independent samples, P value by 2-sided t-test for end point tumor volume. b, Dot plot showing the CD45− gating of YUMM1.7-OVA tumor cells. c–d, Representative histogram showing the expression of H2Db (c) or OVA-H2Kb (d) in YUMM1.7-OVA shFluc or shLimit tumor cells. e–f, Statistical results of H2Db expression (e) or OVA-H2Kb expression (f) in YUMM1.7-OVA shFluc or shLimit tumor cells. mean ± SD, n = 5 biological independent samples, P value by 2-sided t-test. Source data are provided.
Extended Data Fig. 6 | LIMIT cis-activates GBPss to boost MHC-I and tumor immunity.

a–b, Fold changes of Limit expression upon IFNγ treatment in YUMM1.7 cells (a) or CT26 cells (b) stably carrying shFluc, shLimit a, or shLimit b. mean ± SD, n = 3 biological independent samples, P value by 2-sided t-test.

c, A375 cells were transfected with LIMIT cDNA for 24 hours. RNA levels of indicated genes were determined by qRT-PCR. mean ± SD, n = 3 biological independent samples, P value by 2-sided t-test.

d, RPKM of Gbp family members upon IFNγ treatment in B16 cells (GSE99299). mean ± SD, n = 3 biological independent samples.

e, A375 shFluc or shLIMIT cells were overexpressed with GBP1 (GBP1OE), and treated with IFNγ for 48 hours. Surface expression of HLA-ABC or PD-L1 were determined by FACS. mean ± SD, n = 3 biological independent samples, P value by 2-sided t-test.

f, A375 shFluc or shLIMIT cells were overexpressed with GBP1 (GBP1OE), and treated with IFNγ for 24 hours. RNA levels of IRF1 were determined by qRT-PCR. mean ± SD, n = 3 biological independent samples, P value by 2-sided t-test.

g, Dot plots of intra-tumoral CD8+ T cell infiltration and activation in the YUMM1.7 tumors carrying shFluc, shLimit, shGbp2, or shLimit plus shGbp2. h–i, Correlations between GBP1-5 and LIMIT (h) or MHC-I (i) in human melanoma datasets. P value by 2 sided linear regression.

j, Cancer patients having received ICB were divided into low and high GBP groups (bottom 15% vs top 15%). The response rates to ICB were calculated as the percentages of partial response (PR) plus complete response (CR). P value by Chi-square test. Patients were from 4 cohorts.

k, Survival plot of patients with melanoma. Based on the expression levels of GBP1-5, patients were divided into high (top 50%) and low (bottom 50%) groups. P value by 2 sided log-rank test. Source data are provided.
Extended Data Fig. 7 | GBPs activate HSF1 to stimulate MHC-I expression. a, Prediction of potential transcription factors targeting HLA-ABC, TAP1, HSPA5 and CALR. 8 shared transcription factors were altered by IFNγ in A375 cells (GSE99299). b, ChIP-seq results of STAT1 (Hela-S3 cells treated with IFNγ) or HSF1 (HepG2 cells in basal condition) derived from ENCODE at UCSC. The enrichment of STAT1 or HSF1 in the promoters of MHC-I related genes are shown. c, A375 cells were treated with multiple proteostasis stressors and KRIBB11. Surface expression of HLA-ABC was determined 48 hours after treatment. mean ± SD, n = 3 biological independent samples, P value by 2-sided t-test. d, Dot plots of IFNγ+CD8+ T cells or TNFα+CD8+ T cells in MC38 tumors carrying shFluc, shGbp2, shHsf1, and shGbp2 plus shHsf1. Source data are provided.
Extended Data Fig. 8 | HSF1 drives MHC-I expression and tumor immunity. a, B16-OVA cells were treated with IFNγ in the presence or absence of KRI8B11 for 48 hours. Cell surface expression of OVA-H2-Kb was determined by FACS. mean ± SD, n = 4 biological independent samples, P value by 2-sided t-test. b, B16-OVA cells were pretreated with IFNγ in the presence or absence of KRI8B11 for 48 hours, then cultured with OT-1 T cells. Cell death was determined by 7-AAD staining in the CD45- tumor cells. mean ± SD, n = 3 biological independent samples, P value by 2-sided t-test. c, MHC-I (H2-Dd) surface staining of CT26 shFluc or shHsf1 cells treated with IFNγ for 48 hours. mean ± SD, n = 3 biological independent samples, P value by 2-sided t-test. d, YUMM1.7 shFluc or shHsf1 cells were treated with IFNγ in the presence or absence of KRI8B11 for 48 hours. Surface expression of MHC-I (H2-Dd) was determined by FACS. mean ± SD, n = 3 biological independent samples, P value by 2-sided t-test. e, Dot plots of CD3+, Ki67+, IFNγ+, and TNFα+ T cells in YUMM1.7 shFluc or shHsf1 tumors. f, Tumor growth curve of CT26 shFluc or shHsf1 tumors in BALB/c mice. mean ± SD, n = 6 animals, P value by 2-sided t-test for end point tumor volume. g, Percentages of CD3+, IFNγ+, TNFα+, and Ki67+ T cells in CT26 shFluc or shHsf1 tumors. mean ± SD, n = 5 biological independent samples, P value by 2-sided t-test. h, Dot plots of CD3+, Ki67+, IFNγ+, and TNFα+ T cells in CT26 shFluc or shHsf1 tumors. Source data are provided.
**Extended Data Fig. 9 | LIMIT-GBP-HSF1 axis drives MHC-I and tumor immunity and immunotherapy.**

**a-b.** HSF1 signaling genes correlated with MHC-I expression (a) or CD8+ T cell infiltration (b) in Pan-Cancer (TCGA, PANCAN), melanoma (TCGA, SKCM) or sarcoma (TCGA, SARC). P value by 2-sided t-test. The minima, 25% percentile, median, 75% percentile, maxima for each blot are (a) (31.53, 40.0075, 42.87, 45.4925, 53.58), (32.02, 41.1175, 43.66, 46.205, 55), (31.82, 39.785, 43.16, 46.31, 50.39), (33.35, 42.215, 44.53, 47.55, 53.41), (32.9, 38.88, 41.19, 43.21, 47.55), (39.33, 44.75, 46.57, 48.4, 51.3); (b) (3.75, 15.6825, 23.6, 30.48, 49.23), (5.47, 19.5875, 24, 29.1525, 45.42), (7.711, 15.7925, 23.415, 29.7395, 39.722), (4.9254, 18.95, 27.857, 34.862, 47.79), (7.7582, 14.727, 18.553, 22.16, 36.084), (15.024, 22.149, 29.1, 35.582, 43.432). 

**c.** Survival plots of human melanoma patients (TCGA, SKCM). Based on the expression of HSF1 signaling genes, patients were divided into high (n = 150 patients) and low (n = 150 patients) groups. P value by 2-sided log-rank test. 

**d-e.** Single cell RNA-seq derived cell clusters pre- or post- anti-PD-1 therapy in human skin basal cell carcinoma. Two malignant clusters are denoted by color and show different sensitivities to PD-1 blockade. Based on the transcript levels of GBP1, human Pan-Cancers were divided into high (top 10%) and low (bottom 10%) groups. HSF1 target gene transcripts were plotted. 

**f.** Proteomics analysis in melanoma patients having received ICB. Protein expression of GBPs, HSF1 signaling genes, and HLA-ABC were compared in responders (R, n = 40 patients) and non-responders (NR, n = 27 patients). P value by 2-sided t-test. 

**g.** Based on the transcript levels of GBP1, human Pan-Cancers were divided into high (top 10%) and low (bottom 10%) groups. HSF1 target gene transcripts were plotted. Source data are provided.
Extended Data Fig. 10 | Scheme showing how LIMIT-GBP-HSF1 axis affects MHC-I and tumor immunity. Cancer cells (or APCs) express LIMIT in response to IFNγ, thereby locally promoting the transcription of GBPs. GBPs interact with HSP90 and release HSP90-decoyed HSF1, resulting in HSF1 activation. Activated HSF1 stimulates the transcription of MHC-I and MHC-I related genes. MHC-I machinery mediates TAA-recognition and T cell activation, eliciting antitumor immune response.
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- □ □ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- □ □ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- □ □ The statistical test(s) used AND whether they are one- or two-sided
  *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
- □ □ A description of all covariates tested
- □ □ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- □ □ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- □ □ For null hypothesis testing, the test statistic (e.g. t, F, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  *Give P values as exact values whenever suitable.*
- □ □ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- □ □ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- □ □ Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

**Data collection**

BD LSRII or LSRII Fortessa flow cytometry was used to run samples and data was acquired and analyzed by BD FACS Diva software (Ver.8.0.1).

**Data analysis**

Flow cytometry data were analyzed with BD FACS Diva software (Ver.8.0.1)

GraphPad Prism (Ver.8.0.0) were used for data analysis.

For manuscripts utilizing custom algorithms or software that are not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The RNA-seq data (GSE99299) and processed single cell data (GSE123814) were obtained from Gene Expression Omnibus (GEO). The MS proteomics data (PXD006003) were obtained from PRIDE repository. The TCGA cancer datasets were obtained from UCSC Xena (http://xena.ucsc.edu/). The RNA-seq data and clinical information for ICB clinical trials were provided by the respective corresponding authors. All raw data supporting the findings of this study are available from the corresponding author on request. Source data are provided with this paper.
Field-specific reporting

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For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
No sample size calculation was done either for in vivo or in vitro studies. For in vivo studies, n=5-10 mice per group is sufficient to detect meaningful biological differences with good reproducibility. For in vitro studies, all experiments were replicated at least for 3 independent biological samples. We determined the sample size to be sufficient based on our experience and previously studies on tumor and cell models.

Data exclusions
No data were excluded from the manuscript.

Replication
As reported in the figure legends, the findings were reliably reproduced.

Randomization
For in vivo experiments, animals were randomized based on tumor burden before they were assigned into different treatment groups, to make sure the starting tumor burden in different treatment groups was similar before treatment. All groups were age and sex matched. For in vitro studies, cells were seeded, cultured and treated in triplicate wells and analyzed equally, therefore, no randomization were applied for in vitro experiments.

Blinding
Preclinical experiments were not performed in a blinded manner as the investigator needed to know the treatment groups in order to complete the study. All data were acquired and analyzed by software with objective standard, thus blinding was not relevant to the study.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☑ | Antibodies |
| ☑ | Eukaryotic cell lines |
| ☑ | Palaeontology and archaeology |
| | Animals and other organisms |
| | Human research participants |
| | Clinical data |
| | Dual use research of concern |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| | Flow cytometry |
| | MRI-based neuroimaging |

Antibodies

Antibodies used for flow cytometry: 1:50: Anti-CD45 (BD Biosciences), anti-CD90 (S3-19.1, BD Biosciences), anti-CD3 (145-2C11, BD Biosciences), anti-CD4 (RM4-5, BD Biosciences), and anti-CD8 (3B6.7, BD Biosciences) anti-Ki67 (B56, BD Biosciences), anti-TNF [MP5-XT22, BD Biosciences], anti-IFN [XM61.2, BD Biosciences], anti-IFN-G (G46-2.6, BD Biosciences), anti-H2-Dd (H-2Dd, BD Biosciences), anti-H2-Dd (34-2-12, BD Biosciences), anti-OVA H-2 Kb (eBio25-01.16, eBio25-01.16), and anti-CD11 (M1/70, BD Biosciences).

Antibodies for western blotting: anti-Human GSK3β (Santa Cruz, 166960, 1:500), anti-HSF1 (CST, 12972, 1:1000), anti-Phospho-HSF1 (Abcam, 76076, 1:1000), anti-GSK3β (CST, 11854, 1:1000), anti-HSP90 (CST, 4877, 1:1000), anti-HSPA5 (CST, 3177, 1:1000), anti-STAT1 (CST, 9172, 1:1000), anti-Phospho-STAT1 (CST, 9167, 1:1000), anti-RNA (CST, 9422, 1:1000), anti-BCR1 (CST, 2870, 1:1000), anti-CDK4 (CST, 12790, 1:1000), anti-GAPDH (Proteintech, 60004, 1:5000), anti-HLA-ABC (W6/32, Novus Biologicals, 64775, 1:1000).

Antibodies for CHIP (1:100): Normal Rabbit IgG (CST, 2729), anti-HSF1 (CST, 12972).

For in vivo experiments: anti-PD-L1 (InvivoMAb, 1DF-262) and control antibody (InvivoMAb, LTF-2)

Validation

All antibodies were well-recognized clones widely used in the field and purchased from reputable vendors. Each antibody used has been validated for its utilized purchase by the manufacturer, and this information is available on the manufacturer website. These antibodies are further validated internally and routinely used in our lab.
**Eukaryotic cell lines**

Policy information about cell lines

Cell line source(s)

Human melanoma cell line A375 (CRL-1619), mouse melanoma cell lines, B16-F0 (CRL-6322) and YUMM1.7 (CRL-3362), mouse colon cancer cell line CT26 (CRL-2638), and 293T (CRL-3216) were purchased from the American Type Culture Collection (ATCC). Mouse colon cancer cell line MC38 was used previously in the Zou laboratory (ref. 23, 48). B16-OVA cells were established as previously reported (ref. 42). A375 STAT3 KO, A375 GBP1-S KO, and A375 LIMP promoter deletion cell lines were generated in this study.

Authentication

Cell lines were not authenticated.

Mycoplasma contamination

All cell lines in our laboratory are routinely tested for mycoplasma contamination and cells used in this study are negative for mycoplasma.

Commonly misidentified lines

(See ICCLAC register)

No cell line used in the paper is listed in ICCLAC database.

**Animals and other organisms**

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Six- to eight-week-old female NSG (NOD.Cg-Fkdcsclid12gtrtm1Wj/Stz, Stock# 00557), C57BL/6 (C57BL/6J, Stock# 000664), BALB/c (BALB/c, Stock# 000651), and OT-1 (C57BL/6-Tg(TcrαTcrβ)110Mjb/J, Stock# 003831) mice were obtained from the Jackson Laboratory. All mice were maintained under pathogen-free conditions. The animal room has a controlled temperature (18-23°C), humidity (40-60%), and a 12 light/12 dark cycle.

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve samples collected from field.

Ethics oversight

Animal studies were conducted under the approval of the Institutional Animal Care and Use Committee at the University of Michigan (PRO000008278).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

**Flow Cytometry**

Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-PE-TC1).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage [with statistics] is provided.

Methodology

Sample preparation

Cells were trypsinized and washed 1 time with MACS buffer (PBS, 2% FBS, 1mM EDTA). Surface staining was performed by adding antibodies to the cell suspension in 50 μl MACS buffer. After incubating for 30 minutes, cells were washed 1 time with MACS buffer and analyzed on BD Fortessa flow cytometer.

To quantify intratumoral T cells and T cell effector cytokine expression, single-cell suspensions were prepared from fresh tumor tissues by physically passing through 100 μm cell strainers. Immune cells were enriched by density gradient centrifugation. For cytokine staining, intratumoral immune cells were incubated in RPMI culture medium containing PMA (5 ng/ml), ionomycin (500 ng/ml), brefeldin A (1:1000), and Monensin (1:1000) at 37 °C for 4 hours. Anti-CD45-APC (30-F11), anti-CD90 (53-2.1), anti-CD3 (145-2C11), anti-CD4 (RM4-5), and anti-CD8 (53-6.7) antibodies were added for 20 minutes for surface staining. The cells were then washed and resuspended in 1 ml of freshly prepared Fix/Perm solution (BD Biosciences) at 4 °C overnight. After being washed with Perm/Wash buffer (BD Biosciences), the cells were stained with anti-Ki67 (B56), anti-TNF (MP6-XT22), and anti-IFNγ (XM56.1) for 30 minutes, washed, and fixed in 4% formaldehyde (Sigma Aldrich).

Instrument

Data collection and analysis was performed on a LSR II equipped with four lasers or a BD Fortessa equipped with four lasers (BD Biosciences).

Software

All data were analyzed with FACS DIVA software Ver.8.0.1 (BD Biosciences).

Cell population abundance

When cells were sorted or enriched, the purity was confirmed by flow cytometry and in each case the purity was above 90%.

Gating strategy

The cells were gated on FSC-A/SSC-A basis on the location known to contain lymphocytes. Doubles were excluded based on
Gating strategy

FSC-A/FSC-H gating, CD45+ cells were gated for analysis of immune cells. Endogenous T cells were gated on CD45+CD3+CD8+ and analyzed for phenotype and cytokine production.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.