

Supplementary Information for
Leukocyte cell-derived chemotaxin 2 is an antiviral regulator acting through the proto-oncogene MET

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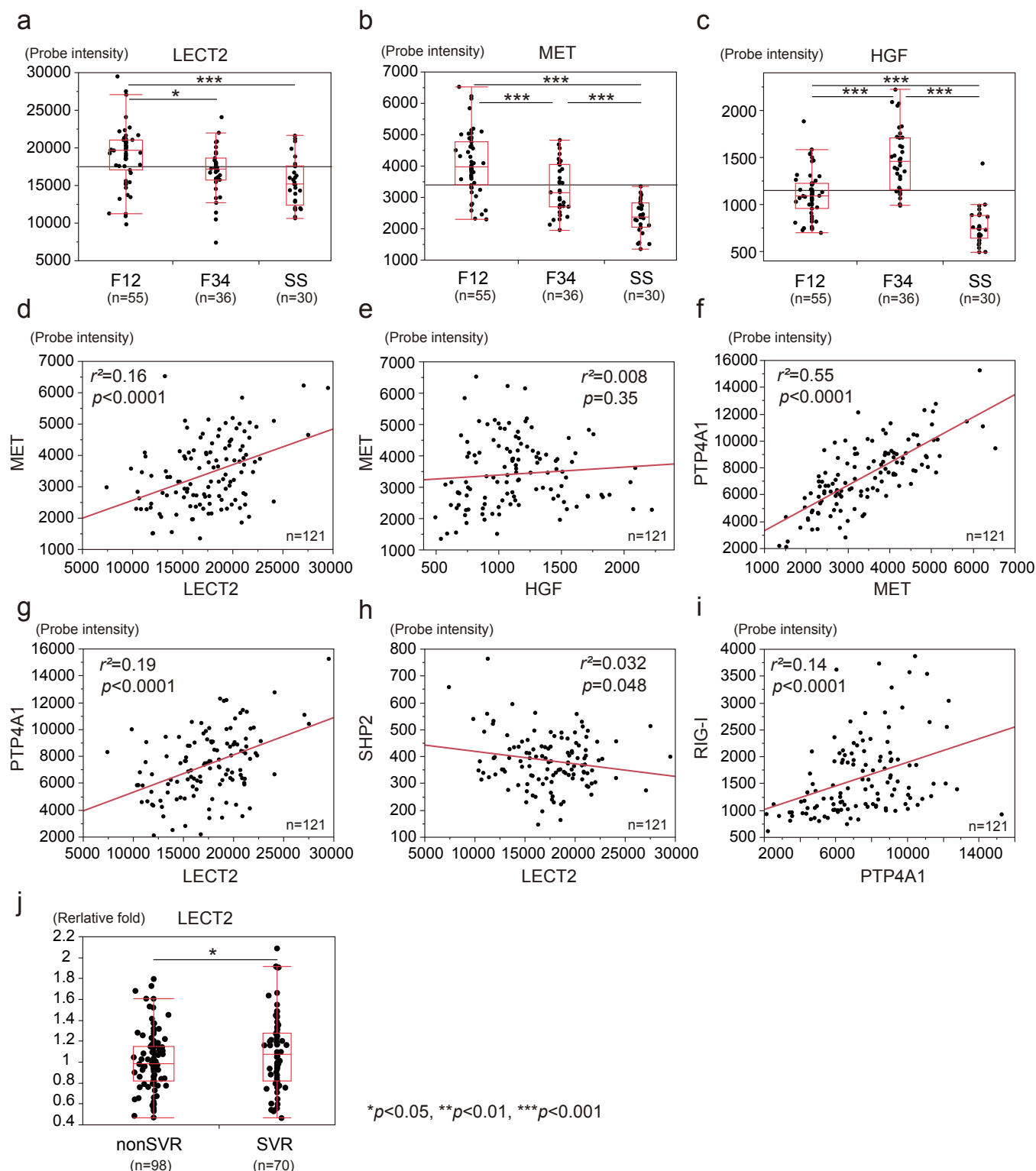
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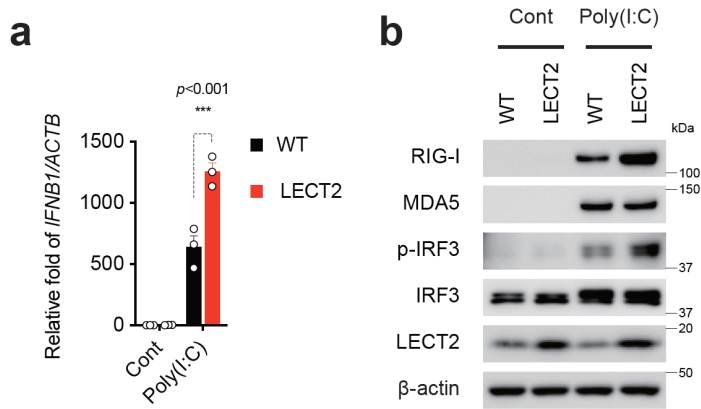
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Supplementary Figure 1: mRNA levels in patients with chronic liver disease.

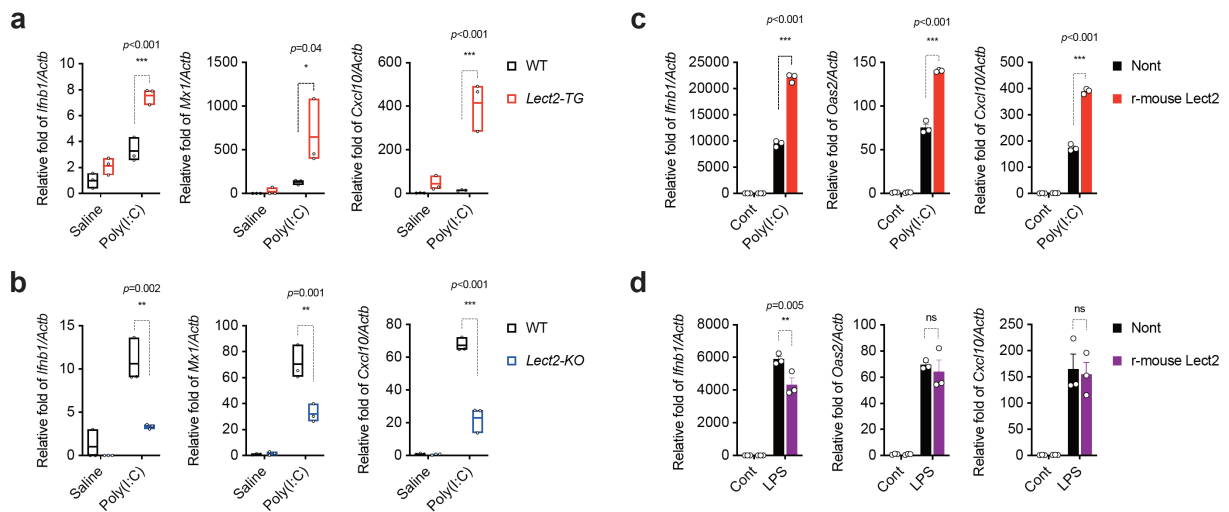
mRNA expression of *LECT2*, *MET*, *HGF*, *PTP4A1*, *SHP2* and *RIG-I* were evaluated using liver tissue obtained from 91 CHC patients (F12, n = 55; F34, n = 36) before they received PEG-

IFNa-2b and RBV combination therapy and 30 patients with early fibrosis (F12), advanced fibrosis (F34) and simple steatosis (SS). Boxplots: center line, median; box limits, 25 to 75th percentiles; whiskers, min to max. **(a-c)** Data shown are means $\pm$ SEM by two-way ANOVA with Tukey's multiple comparison test. **(d-i)** Simple linear regression analysis (one-sided) was performed between the two values. **(j)** Data shown are means $\pm$ SEM by two-way ANOVA with unpaired Student's t-test.



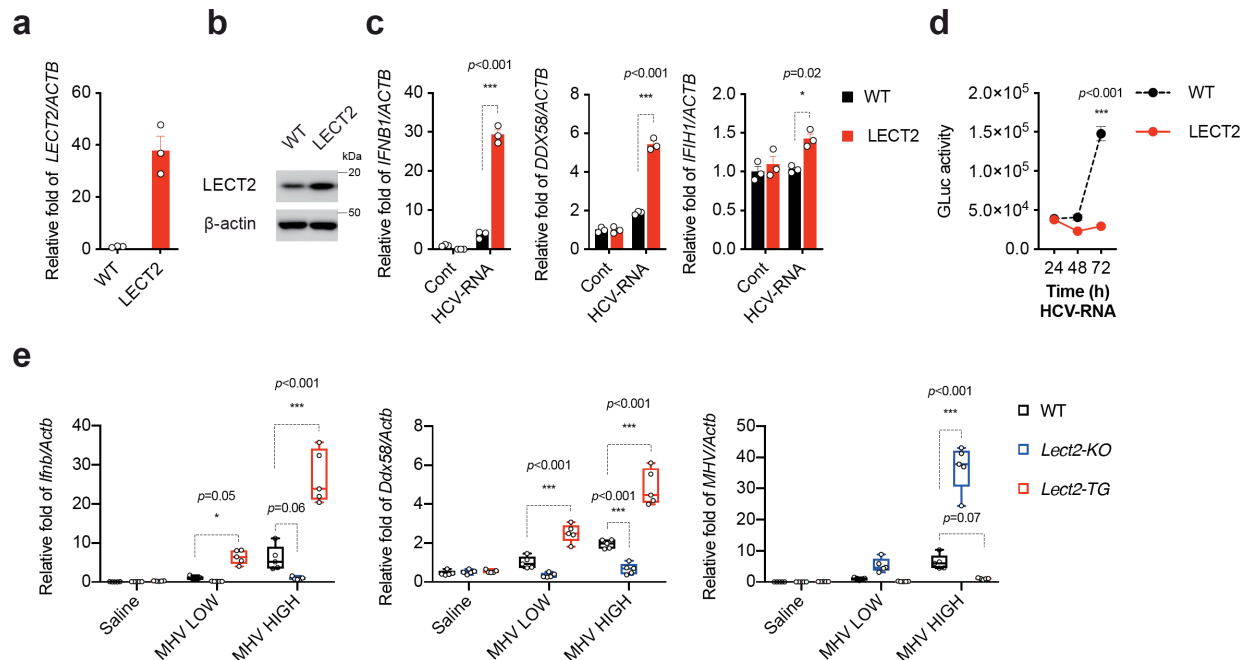
Supplementary Figure 2: LECT2 enhances innate immune responses induced by Poly(I:C).

(a, b) HepG2-WT and HepG2-LECT2 cells were transfected with Poly(I:C). **(a)** qRT-PCR analysis of *IFNB1* in HepG2-WT and HepG2-LECT2 cells at 12 h after mock (Cont) or Poly(I:C). transfection. Results were normalised to those of *ACTB*. Data shown are means of 3 technical replicates $\pm$ SEM. *** $p < 0.001$ by two-way ANOVA with Tukey's multiple comparison test. **(b)** Immunoblot analysis of RIG-I, MDA5, p-IRF3, IRF3, LECT2, and β -actin in HepG2-WT and HepG2-LECT2 cells at 6 h after mock (Cont) or HCV-RNA transfection. Source data are provided as a Source Data file.



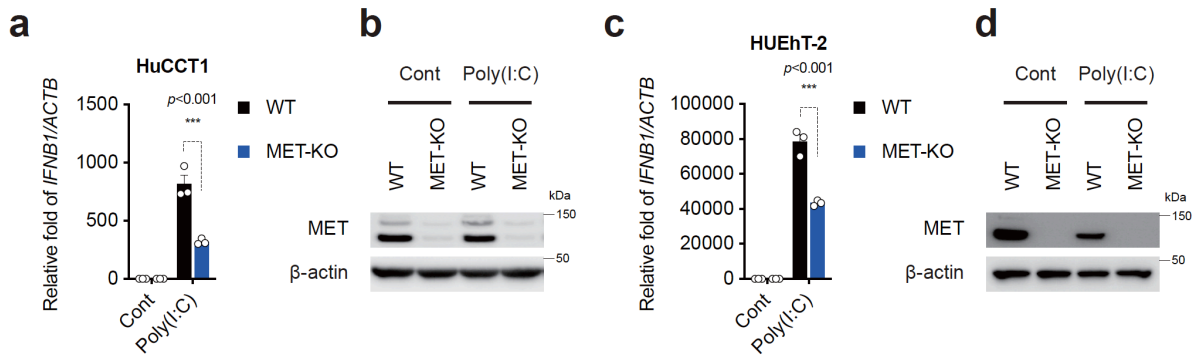
Supplementary Figure 3: Lect2 enhances innate immune responses induced by Poly(I:C) in mice and mouse primary hepatocytes.

(a, b) WT, *Lect2-TG*, and *Lect2-KO* mice were intravenously injected with saline or Poly(I:C). (a) qRT-PCR analysis of *Ifnb1*, *Mx1*, and *Cxcl10* in the liver of WT and *Lect2-TG* mice at 12 h after injection. Results were normalised to those of *Actb*. Data shown are means of 3 biological replicates $\pm$ SEM. *** $p < 0.001$, * $p = 0.04$ by two-way ANOVA with Tukey's multiple comparison test. Boxplots: center line, median; box limits, 25 to 75th percentiles; whiskers, min to max. (b) qRT-PCR analysis of *Ifnb1*, *Mx1*, and *Cxcl10* in the liver of WT and *Lect2-KO* mice at 12 h after injection. Results were normalised to those of *Actb*. Data shown are means of 3 biological replicates $\pm$ SEM. *** $p < 0.001$, ** $p = 0.002$ (*Ifnb1/Actb*), ** $p = 0.002$ (*Mx1/Actb*) by two-way ANOVA with Tukey's multiple comparison test. Boxplots: center line, median; box limits, 25 to 75th percentiles; whiskers, min to max. (c, d) Mouse primary hepatocytes were treated with r-mouse Lect2 for 1 h and then transfected with Poly(I:C) or treated with LPS. (c) qRT-PCR analysis of *Ifnb1*, *Oas2*, and *Cxcl10* in cells at 12 h after mock (Cont) or Poly(I:C) transfection in the presence or absence (Nont) of r-mouse Lect2. Results were normalised to those of *Actb*. Data shown are means of 3 technical replicates $\pm$ SEM. *** $p < 0.001$ by two-way ANOVA with Tukey's multiple comparison test. (d) qRT-PCR analysis of *Ifnb1*, *Oas2*, and *Cxcl10* in cells at 12 h after non-treatment (Cont) or LPS treatment in the presence or absence (Nont) of r-mouse Lect2. Results were normalised to those of *Actb*. Data shown are means of 3 technical replicates $\pm$ SEM. ** $p = 0.005$, not significant (ns), by two-way ANOVA with Tukey's multiple comparison test. Source data are provided as a Source Data file.



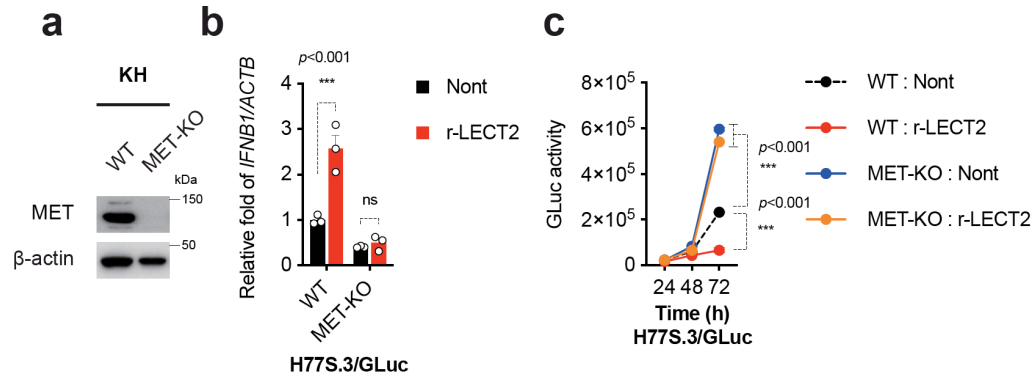
Supplementary Figure 4: LECT2 suppresses HCV and MHV replication.

(a) qRT-PCR analysis of *LECT2* in KH-WT and KH-LECT2 cells. Results were normalised to those of *ACTB*. (b) Immunoblot analysis of *LECT2* and β -actin in KH-WT and KH-LECT2 cells. (c, d) KH-WT and KH-LECT2 cells were transfected with HCV-RNA (H77S.3/GLuc-RNA). (c) qRT-PCR analysis of *IFNB1*, *DDX58*, and *IFIH1* in KH-WT and KH-LECT2 cells at 12 h after mock (Cont) and HCV-RNA transfection. Results were normalised to those of *ACTB*. Data shown are means of 3 technical replicates $\pm$ SEM. *** $p < 0.001$, * $p = 0.02$ by two-way ANOVA with Tukey's multiple comparison test. (d) GLuc activity in KH-WT and KH-LECT2 cells at 12 h after HCV-RNA transfection. Data shown are means of 3 technical replicates $\pm$ SEM. *** $p < 0.001$ by two-way ANOVA with Tukey's multiple comparison test. (e) WT, *Lect2-TG*, and *Lect2-KO* mice were infected with 5 pfu (LOW) or 100 pfu (HIGH) MHV via intraperitoneal injection. qRT-PCR analysis of *Ifnb1*, *Ddx58*, and MHV in the liver of WT, *Lect2-TG*, and *Lect2-KO* mice at 7 days after saline injection or MHV infection. Results were normalised to those of *Actb*. Data shown are means of 5 biological replicates $\pm$ SEM. *** $p < 0.001$, * $p = 0.05$ by two-way ANOVA with Tukey's multiple comparison test. Boxplots: center line, median; box limits, 25 to 75th percentiles; whiskers, min to max. Source data are provided as a Source Data file.



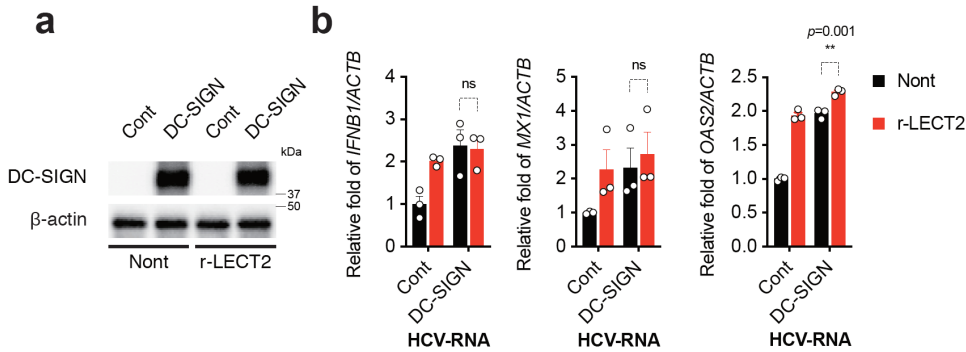
Supplementary Figure 5: MET has an important role in innate immune responses in HuCCT1 and HUEhT-2 cells.

(a, b) HuCCT1-WT and HuCCT1-MET-KO cells were transfected with Poly(I:C). (a) qRT-PCR analysis of *IFNB1* in HuCCT1-WT and HuCCT1-MET-KO cells at 12 h after mock (Cont) or Poly(I:C) transfection. Results were normalised to those of *ACTB*. Data shown are means of 3 technical replicates $\pm$ SEM. *** $p < 0.001$ by two-way ANOVA with Tukey's multiple comparison test. (b) Immunoblot analysis of MET and β -actin in HuCCT1-WT and HuCCT1-MET-KO cells at 12 h after mock (Cont) or Poly(I:C) transfection. (c, d) HUEhT-2-WT and HUEhT-2-MET-KO cells were transfected with Poly(I:C). (c) qRT-PCR analysis of *IFNB1* in HUEhT-2-WT and HUEhT-2-MET-KO cells at 12 h after mock (Cont) or Poly(I:C) transfection. Results were normalised to those of *ACTB*. Data shown are means of 3 technical replicates $\pm$ SEM. *** $p < 0.001$ by two-way ANOVA with Tukey's multiple comparison test. (d) Immunoblot analysis of MET and β -actin in HUEhT-2-WT and HUEhT-2-MET-KO cells at 12 h after mock (Cont) or Poly(I:C) transfection. Source data are provided as a Source Data file.



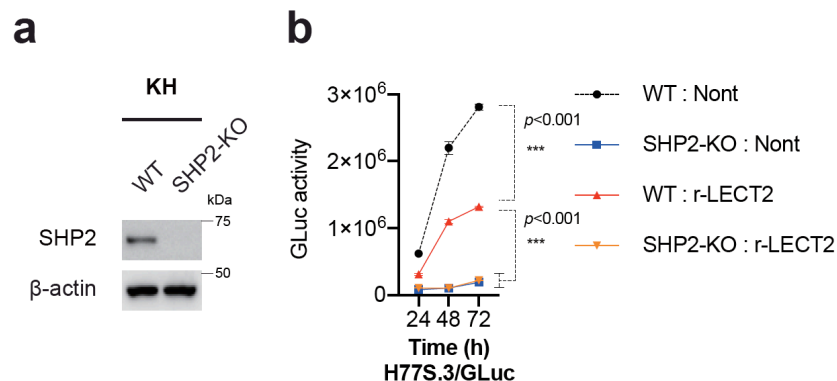
Supplementary Figure 6: MET is essential for LECT2-mediated innate immune responses in KH cells.

(a) Immunoblot analysis of MET and β -actin in KH-WT and KH-MET-KO cells. (b, c) KH-WT and KH-MET-KO cells were treated with r-LECT2 at a concentration of 50 ng/mL for 1 h and then transfected with H77S.3/GLuc-RNA. (b) qRT-PCR analysis of *IFNB1* in KH-WT and KH-MET-KO cells at 12 h after H77S.3/GLuc-RNA transfection in the presence or absence (Nont) of r-LECT2. Results were normalised to those of *ACTB*. Data shown are means of 3 technical replicates $\pm$ SEM. *** $p < 0.001$, not significant (ns) by two-way ANOVA with Tukey's multiple comparison test. (c) GLuc activity in KH-WT and KH-MET-KO cells at 24, 48, and 72 h after H77S.3/GLuc-RNA transfection in the presence or absence (Nont) of r-LECT2. Data shown are means of 3 technical replicates $\pm$ SEM. *** $p < 0.001$ by two-way ANOVA with Tukey's multiple comparison test. Source data are provided as a Source Data file.



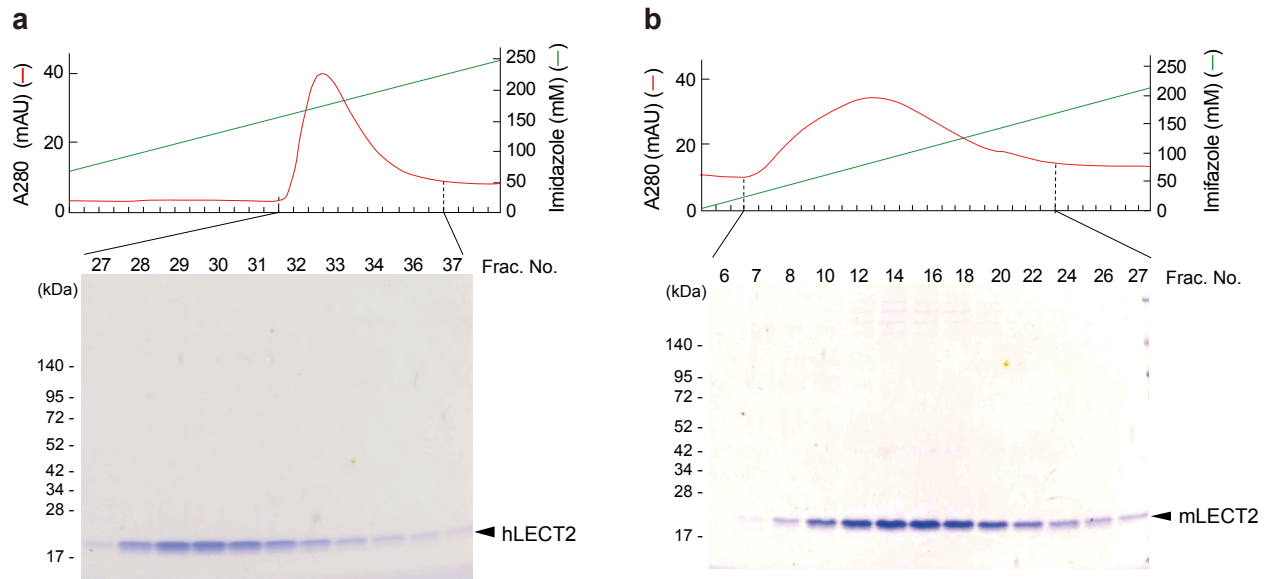
Supplementary Figure 7: DC-SIGN does not affect LECT2-mediated innate immune responses.

(a, b) HepG2 cells were transfected with the DC-SIGN plasmid. At 48 h after transfection, the cells were treated with 50 ng/mL r-LECT2 for 1 h and then transfected with HCV-RNA. (a) Immunoblot analysis of DC-SIGN and β -actin in these cells at 12 h after HCV-RNA transfection in the presence or absence (Nont) of r-LECT2. (b) qRT-PCR analysis of *IFNB1*, *MX1*, and *OAS2* in these cells at 12 h after HCV-RNA transfection in the presence or absence (Nont) of r-LECT2. Results were normalised to those of *ACTB*. Data shown are means of 3 technical replicates $\pm$ SEM. **p = 0.001, not significant (ns) by two-way ANOVA with Tukey's multiple comparison test. Source data are provided as a Source Data file.



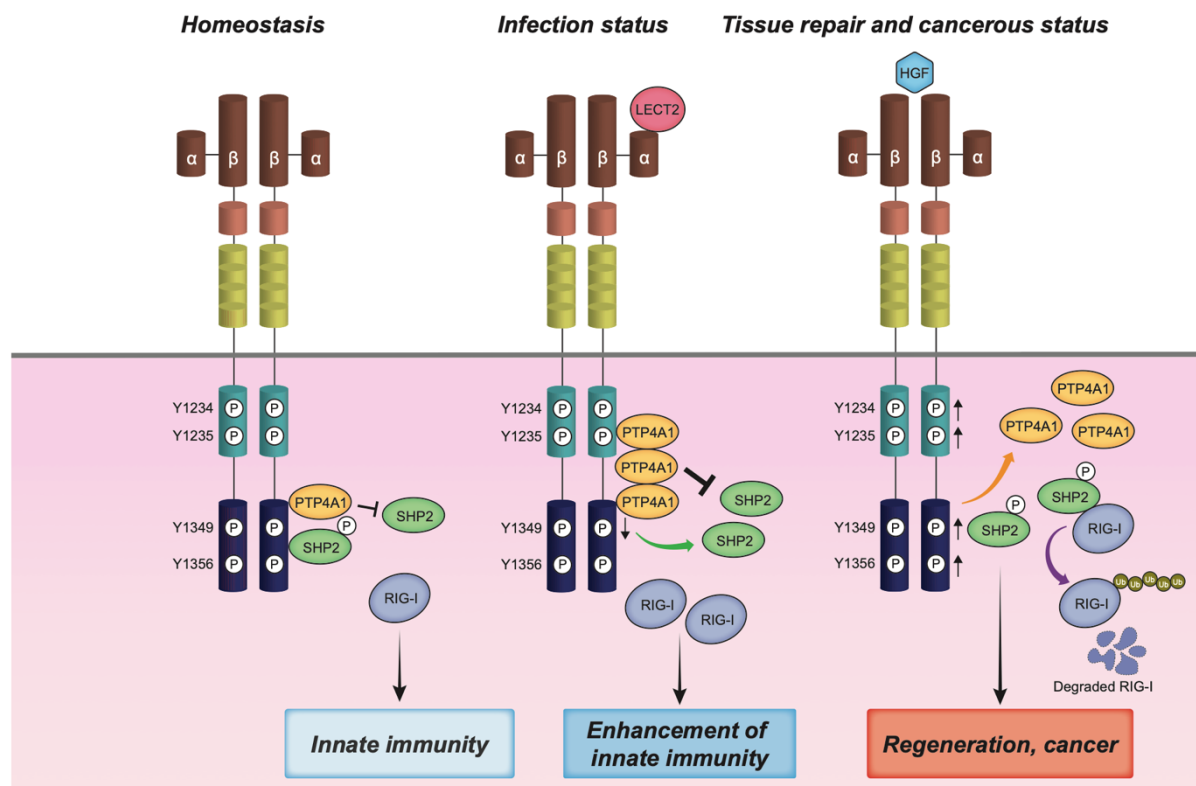
Supplementary Figure 8: LECT2 does not affect HCV replication in the absence of SHP2.

(a) Immunoblot analysis of SHP2 and β-actin in KH-WT and KH-SHP2-KO cells. **(b)** KH-WT and KH-SHP2-KO cells were treated with 50 ng/mL r-LECT2 for 1 h and then transfected with H77S.3/GLuc-RNA. GLuc activity in KH-WT and KH-SHP2-KO cells at 24, 48, and 72 h after H77S.3/GLuc-RNA transfection in the presence or absence (Nont) of r-LECT2. Data shown are means of 3 technical replicates ± SEM. *** $p < 0.001$ by two-way ANOVA with Tukey's multiple comparison test. Source data are provided as a Source Data file.



Supplementary Figure 9: Preparation of recombinant LECT2.

(a, b; upper) Human and mouse recombinant LECT2 were eluted using a concentration gradient of imidazole. Fractions containing LECT2 were applied and eluted using a heparin column to remove imidazole. **(a, b; lower)** Coomassie brilliant blue staining for recombinant LECT2 in each fraction.



Supplementary Figure 10: Schematic representation of the role of LECT2 in innate immune response. LECT2 promotes the recruitment of PTP4A1 to MET, and PTP4A1 dephosphorylates SHP2 under ligand-free conditions. PTP4A1 and SHP2 have antagonistic effects on MET, and their competition for binding MET maintains a normal homeostatic balance. When cells are infected with virus, LECT2 becomes the predominant ligand for MET, tipping the balance toward PTP4A1 engagement and resulting in an enhancement of RIG-I responses. In contrast, in tissue repair and cancerous states, HGF is the predominant ligand for MET, resulting in a suppression of the innate immune response and enhancement of proliferative signals.