**Expanded View Figures**

**A**

| Clone | THP-1 WT | ΔRIG-I 1 | ΔMDA5 2 | ΔLGP2 3 |
|-------|----------|----------|----------|----------|
|       | sIC      | sIC      | sIC      | sIC      |
|       | sIA      | sIA      | sIA      | sIA      |

ADAR1 (short exp.)

ADAR1 (long exp.)

Actin

**B**

| IFN-I | HEK293 | THP-1 |
|-------|--------|-------|
| -     | +      | +     |
|        |

ADAR1

MDA5

LGP2

ISG60

Actin

**C**

**D**

**E**

![Figure EV1](image-url)

Figure EV1.
Figure EV1. LGP2 is required for the induction of a type I IFN response upon siRNA-mediated depletion of ADAR1 in HEK293, related to Fig 1.

A PMA-differentiated THP-1 WT, RIG-I-, MDA5-, and LGP2-knockout cells were transfected with siCtrl (siC) or siADAR1 (siA). Protein lysates were prepared 56 h post-transfection and ADAR1 knockdown efficiency was monitored by immunoblot analysis. Data correspond to the biological replicate shown in Fig 1B.

B Expression level and type I IFN inducibility of relevant proteins in HEK293 and THP-1. HEK293 and PMA-differentiated THP-1 cells were treated with or without recombinant type I IFN. Protein lysates were analyzed by SDS-PAGE followed by immunoblotting with the indicated antibodies (n = 3). *, nonspecific band.

C Kinetics of siRNA-mediated depletion of ADAR1 and induction of the type I IFN response in HEK293. HEK293 cells stably expressing FLAG-LGP2 were transfected with an siRNA targeting ADAR1 (siADAR1) and harvested at the indicated time points post-transfection. ADAR1 knockdown and IFIT1 upregulation were monitored by RT-qPCR analysis (using Taqman probes) and normalized to ACTB. Data are means ± s.d. from one experiment.

D HEK293 WT cells were transfected with siADAR1 or a control siRNA (siCtrl) and 8 h later with increasing amounts of a vector encoding FLAG-LGP2. As a control, cells were transfected with 250 ng of an empty vector (EV). Cells were harvested 80 h post siRNA transfection. RT-qPCR analysis was used to monitor IFN-β and IFIT1 expression, ADAR1 knockdown, and LGP2 (DHX58) expression. All transcripts were normalized to ACTB. Data are means ± s.d. from a representative of two biological replicate experiments.

E Cells were treated as in (D). Protein lysates were prepared and analyzed by SDS-PAGE followed by immunoblotting using the indicated antibodies.

Source data are available online for this figure.

Figure EV2. LGP2-deficient cells fail to sense unedited self RNAs, yet maintain the ability to detect viral dsRNAs, related to Fig 1.

A WT and LGP2-knockout (clones 1 and 2) HEK293 cells were transfected with an siRNA targeting ADAR1 (siADAR1) or a control siRNA (siCtrl) and were treated 8 h later with recombinant type I IFN to upregulate RLR expression. Cells were harvested 80 h post siRNA transfection and RT-qPCR analysis was used to monitor IFN-β and IFIT1 expression and ADAR1 knockdown. All transcripts were normalized to ACTB. Data are means ± s.d. from a representative of three biological replicate experiments.

B Cells were treated as in (A). Protein lysates were prepared 80 h post siRNA transfection, followed by SDS-PAGE and immunoblotting using the indicated antibodies (n = 3). siC, siCtrl; siA, siADAR1.

C LGP2-knockout (clones 1 and 2) HEK293 cells were transfected with siADAR1 or siCtrl and 8 h later with a vector encoding FLAG-LGP2 or an empty vector (EV). Cells were harvested 80 h post siRNA transfection and RT-qPCR analysis was used to monitor IFN-β and IFIT1 expression and ADAR1 knockdown. All transcripts were normalized to ACTB. Data are means ± s.d. from a representative of two biological replicate experiments.

D Cells were treated as in (C). Protein lysates were prepared 80 h post siRNA transfection, followed by SDS-PAGE and immunoblotting using the indicated antibodies (n = 2).

E, F WT, LGP2-knockout (clones 1 and 2), and stably expressing FLAG-LGP2 HEK293 cells were transfected with transfection reagent only (LF2000), poly(I:C) (56, 112, 225, or 450 ng in E)), or RNA isolated from HEK293 cells infected with EMCV in the presence of ribavirin (450 or 900 ng in F)). Cells were harvested 16 h post-transfection and RT-qPCR analysis was used to monitor IFN-β and IFIT1 expression. All transcripts were normalized to ACTB. Data are means ± s.d. from a representative of four (E) or three (F) biological replicate experiments.

G MDA5-knockout HEK293 cells stably expressing FLAG-LGP2 or an empty vector (EV) were transfected with increasing amounts (5, 20, 40, 80, or 240 ng/ml) of a vector encoding FLAG-MDA5 WT or FLAG-MDA5 C-499R. As a control, cells were transfected with 240 ng/ml control vector or left untreated. Cells were harvested 24 h post-transfection and RT-qPCR analysis was used to monitor IFN-β and IFIT1 expression. All transcripts were normalized to ACTB. Data are means ± s.d. from a representative of two biological replicate experiments.

Source data are available online for this figure.
Figure EV2 - Stok & Oosenbrug et al.

**A**

HEK293 WT ΔLGP2 clone 1 ΔLGP2 clone 2

**B**

HEK293 WT ΔLGP2 clone 1 ΔLGP2 clone 2

**C**

HEK293 WT ΔLGP2 clone 1 ΔLGP2 clone 2

**D**

ΔLGP2 clone 1 ΔLGP2 clone 2

**E**

HEK293 WT ΔLGP2 clone 1 ΔLGP2 clone 2 FLAG-LGP2

**F**

HEK293 WT ΔLGP2 clone 1 ΔLGP2 clone 2 FLAG-LGP2

**G**

HEK293 MDA5 WT MDA5 G495R MDA5 WT MDA5 G495R

Figure EV2 - Stok & Oosenbrug et al.
Figure EV3. A type I IFN response is unleashed in ADAR1-knockout cells upon expression of LGP2, related to Figs 2 and 3.

A ADAR1-knockout HEK293 cells (clone 2) were cotransfected with an empty vector (EV) or a FLAG-LGP2-encoding vector (LGP2) combined with a vector encoding GFP-tagged ADAR1 p110 or p150. Cells were harvested 48 h post-transfection and the type I IFN response was monitored by measuring IFN-β and IFIT1 transcript expression, relative to ACTB expression, by RT-qPCR. Data are means ± s.d. from a representative of three biological replicate experiments.

B ADAR1-knockout HEK293 cells (clone 2) were transfected as in (A). Protein lysates were analyzed by SDS-PAGE followed by immunoblotting using the indicated antibodies (n = 3).

C MDAS-knockout HEK293 cells, generated in Fig 1D, were transfected with an ADAR1-targeting siRNA (siADAR1) or a control siRNA (siCtrl) and 8 h later with an empty vector (EV) or a vector encoding the indicated WT, truncation, or point mutant(s) of MDAS. Cells were harvested 72 h post-siRNA transfection and RT-qPCR analysis was used to monitor ADAR1 knockdown and MDAS (IFIH2) expression. All transcripts were normalized to ACTB. Data are means ± s.d. from a representative of two biological replicate experiments.

Source data are available online for this figure.

Figure EV4. Loss of ADAR1 inhibits tumor cell growth in an LGP2-dependent manner, related to Fig 5.

A ADARWT patients with concomitant DHX58hyp expression have prolonged survival across multiple cancer types. Hazard ratios and 95% confidence intervals from univariate Cox regression models for DHX58 stratification in ADARWT (left panel) and ADARhyp (right panel) patients from 17 TCGA datasets (sarcoma — SARC, liver — LIHC, esophageal — ESCA, breast — BRCA, bladder — BLCA, endometrial —UCEC, rectal — READ, cervical — CESC, melanoma — SKCM, ovarian — OV, pancreas — PAAD, lung adenocarcinoma — LUAD, stomach — STAD, head and neck — HNSC, clear cell renal cell carcinoma — RENCA, lung squamous — LUSC, colon — COAD). Median cut-off values for both ADAR and DHX58 were used for patient stratification. Dashed lines indicate a hazard ratio of 1. Wald test P values are shown.

B–D CAL27 cells were transduced with doxycycline-inducible shRNAs targeting ADAR1 or GFP (negative control) and subsequently treated with doxycycline and/or transfected with two independent siRNAs targeting LGP2 (siLGP2 #1 or #2) or a control siRNA (siCtrl). (B) Cells were harvested 72 h post-treatment and RT-qPCR analysis was used to monitor knockdown efficiency of ADAR1 and LGP2 (DHX58) upon doxycycline treatment or siRNA transfection, respectively. All transcripts were normalized to ACTB. Data are means ± s.d. from a representative of three biological replicate experiments. (C) Cell confluency was measured every 24 h post-treatment and RT-qPCR analysis was used to monitor knockdown efficiency of ADAR1 and LGP2 (DHX58) upon doxycycline treatment or siRNA transfection, respectively. All transcripts were normalized to ACTB. Data are means ± s.d. from a representative of three biological replicate experiments. (D) Knockdown efficiency of ADAR1 and LGP2 (DHX58) in samples of (C) at 120 h post-treatment was determined as in (B). Data are means ± s.d. from one experiment.
Figure EV4.
Figure EV5. Type I IFN responsiveness and siADAR1-dependent type I IFN induction in various cancer cell lines, related to Figs 5 and 6.

A Endogenous expression level and type I IFN inducibility of relevant proteins in HT29, LIM1215, and CAL27 cells. Cells were treated with or without recombinant type I IFN. Protein lysates were analyzed by SDS-PAGE followed by immunoblotting with the indicated antibodies. *, nonspecific band.

B HT29 cells were transfected with the indicated siRNAs. Cells were harvested 72 h post-transfection and RT-qPCR analysis was used to monitor the type I IFN response (IFN-β, IFIT1, and ISG15 transcripts) and knockdown efficiency of ADAR1 and LGP2 (DHX58). All transcripts were normalized to ACTB. Data are means ± s.d. from a representative of three biological replicate experiments.

C LIM1215 cells were transfected with the indicated siRNAs. Cells were harvested 72 h post-transfection and knockdown efficiency of ADAR1 and LGP2 (DHX58) was analyzed as in (C). Data are means ± s.d. from a representative of three biological replicate experiments.

Source data are available online for this figure.