

Supplementary materials

DDX50 Is a Viral Restriction Factor That Enhances IRF3 Activation

Table S1. Constructs and primers used in the study.

| Plasmids | Description | Reference/Source |
|---|---|------------------------------|
| pCW57-GFP-P2A-MCS | Lentiviral expression plasmid with GFP in MSC1 and P2A skip sequence followed by MCS2 under the control of a CMV promoter, Amp ^r and Puro ^r | Addgene, 71783 |
| | Primer | RE |
| pCW57-GFP-P2A- <i>Ddx50</i> -HA | CGACGCGTATGCCCGGGAAACTCCTCTGG GCAGGATCCTTAAGCGTAATCTGGAACATCGTAT | MluI BamHI This study |
| pCMV-PACK | Packaging plasmid for lentivirus production with HIV Gag, Pol, Rev and Tat under the CMV promoter, Amp ^r | Gift, Dr. H. Laman |
| pCMV-ENV | VSV-G pseudotyped envelope protein under the CMV promoter for lentivirus production, Amp ^r | Gift, Dr. H. Laman |
| pLDT-TetR | Lentiviral expression plasmid constitutively expressing the Tetracycline promoter repressor, Amp ^r and Neo ^r | (Everett et al, 2013) |
| pLDT-MCS | Lentiviral expression plasmid with expression under the control of a Tetracycline inducible CMV promoter, Amp ^r and Puro ^r | (Everett et al, 2013) |
| | Primer | RE |
| pLDT- <i>DDX50</i> -HA | CGGCTAGCATGCCTGGGAAACTCCTCTGG CGGATATCTTAAGCGTAATCTGGAACATCGTATGGGTAG- TCAAAACTCCGTT TGTGGCC | NheI EcoRV This study |
| pLDT- <i>DDX28</i> -HA | CGGATATCATGGCTCTAACGCGGCCGGT CGGATATCTTAAGCGTAATCTGGAACATCGTATGGGTAGGTTGCTT- GGGGCA AAGGCTC | EcoRV EcoRI This study |
| pLDT- <i>Ddx50</i> | CGGCTAGCATGCCCGGGAAACTCCTCTGG CGGATATCTCAGTCAAATTCGGTTTATG | NheI EcoRV This study |
| pCDNA4/TO-nTAP | Mammalian expression plasmid with expression under the control of a Tetracycline inducible CMV promoter, Amp ^r and Zeocin ^r | (Pallett et al, 2019) |
| pMX-CMV-YFP | Retroviral plasmid for viral package signal, transcription and processing, YFP expression and puromycin selectable, Amp ^r | Invitrogen |
| pMX-CMV-YFP-miR30E <i>DDX1</i> clone 1 | tcgagaaggtatattgctgtgacagtgagcgccccgggcaatcaaggaacataatagtgagccacagat cttgaagtccgaggcagtaggcatccgggcaatcaaggaacataatacatctgtggcttactattatgt | This study |
| pMX-CMV-YFP-miR30E <i>DDX1</i> clone 2 | tcgagaaggtatattgctgtgacagtgagcgcgatgtggtctgaagctattaatagtgagccacagat cttgaagtccgaggcagtaggcaagatgtggtctgaagctattaatacatctgtggcttactattaata | This study |
| pMX-CMV-YFP-miR30E <i>LacZ</i> | tcgagaaggtatattgctgtgacagtgagcgACGTCGTATTACAACGTCGTGAtagtgaa- gccacagat cttgaagtccgaggcagtaggcaCCGTCGTATTACAACGTCGTGAtacatctgtggcttca- taTCACGA | This study |

Amp^r, Ampicillin resistance; Puro^r, Puromycin resistance; Neo^r, Neomycin resistance; Zeocin^r, Zeocin resistance; RE, restriction enzyme.

Table S2. Primers for qPCR.

| Primer | Sequence |
|-------------------|----------------------------|
| <i>Ifnb</i> Fwd | CATCAACTATAAGCAGCTCCA |
| <i>Ifnb</i> Rev | TTCAAGTGGAGAGCAGTTGAG |
| <i>Il-6</i> Fwd | GTAGCTATGGTACTCCAGAAG |
| <i>Il-6</i> Rev | ACGATGATGCACTTGCAGAA |
| <i>Cxcl10</i> Fwd | ACTGCATCCATATCGATGAC |
| <i>Cxcl10</i> Rev | TTCATCGTGGCAATGATCTC |
| <i>Isg56</i> Fwd | ACCATGGGAGAGAATGCTGAT |
| <i>Isg56</i> Rev | GCCAGGAGGTTGTGC |
| <i>Nfkbia</i> Fwd | CTGCAGGCCACCACCTACAA |
| <i>Nfkbia</i> Rev | CAGCACCCAAAGTCACCAAGT |
| <i>Gapdh</i> Fwd | ATCAACGACCCCTTCATTGACC |
| <i>Gapdh</i> Rev | CCAGTAGACTCCACGACATACTCAGC |

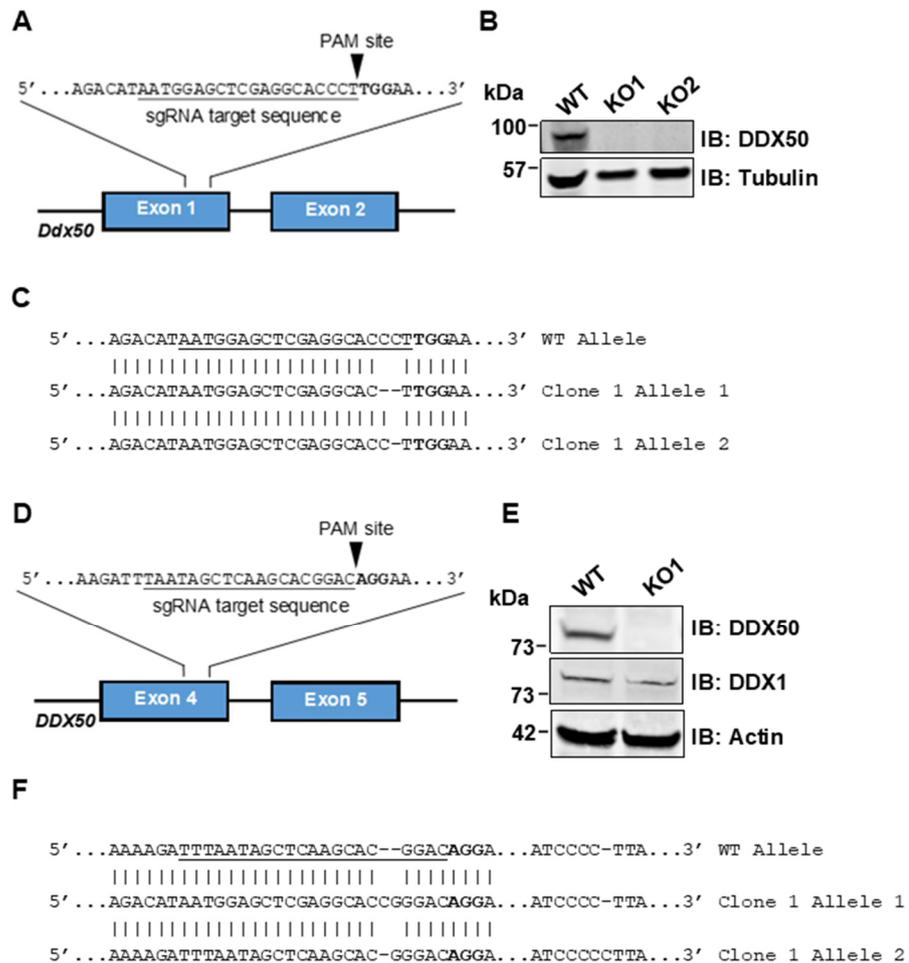


Figure S1. CRISPR-Cas9 mediated knockout of *Ddx50/DDX50* (*RH-III/Guβ*). WT mouse (A-C) fibroblasts and human HEK293Ts (D-F) were transfected with pX459 - sgRNA targeting *Ddx50* exon 1 (A) and *DDX50* exon 4 (D), respectively. Cells were selected in 4 μg/ml (MEFs) or 1 μg/ml (HEK293T) puromycin for 2 weeks before clones were expanded. (A and D) Schematic representation of the genomic target for each mutation. (B and E) SDS-PAGE and immunoblot to assess levels of DDX50. KO, Knockout clone. (C and F) Single allele sequencing of exon 1 and exon 4 for *Ddx50* and *DDX50*, respectively.

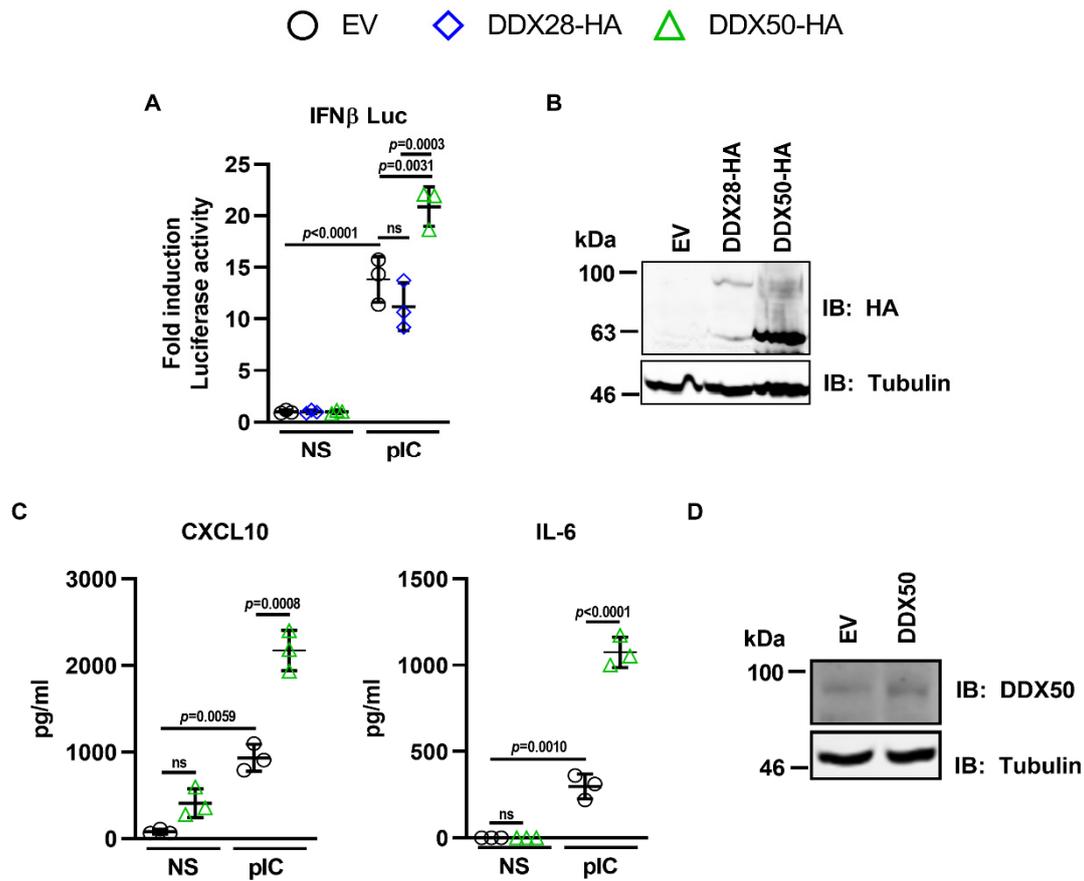


Figure S2. DDX50 overexpression augments nucleic acid sensing. **(A)** The human fibroblast (HF) EV, DDX50-HA or DDX28-HA cell lines were transfected with plasmids encoding Firefly Luciferase under the *Ifn β* promoter or Renilla, and Firefly Luciferase fold induction was analysed following transfection with 5 μ g/ml Poly IC for 6 h. **(B)** Relative levels of DDX28 and DDX50 expression were confirmed by immunoblotting with anti-HA and anti- α -tubulin. Representative of three independent experiments. **(C)** WT MEF EV or DDX50 transduced cell lines were transfected with lipofectamine only or 5 μ g/ml PolyIC for 7 h and the secretion of CXCL10 and IL-6 was measured by ELISA. Representative of at least two independent experiments. For all panels statistical significance was determined by performing a Two-way ANOVA test followed by Tukey's multiple comparison post-hoc test. ns, non-significant. **(D)** Overexpression of DDX50 was confirmed by immunoblotting with anti-DDX50 and anti- α -tubulin.

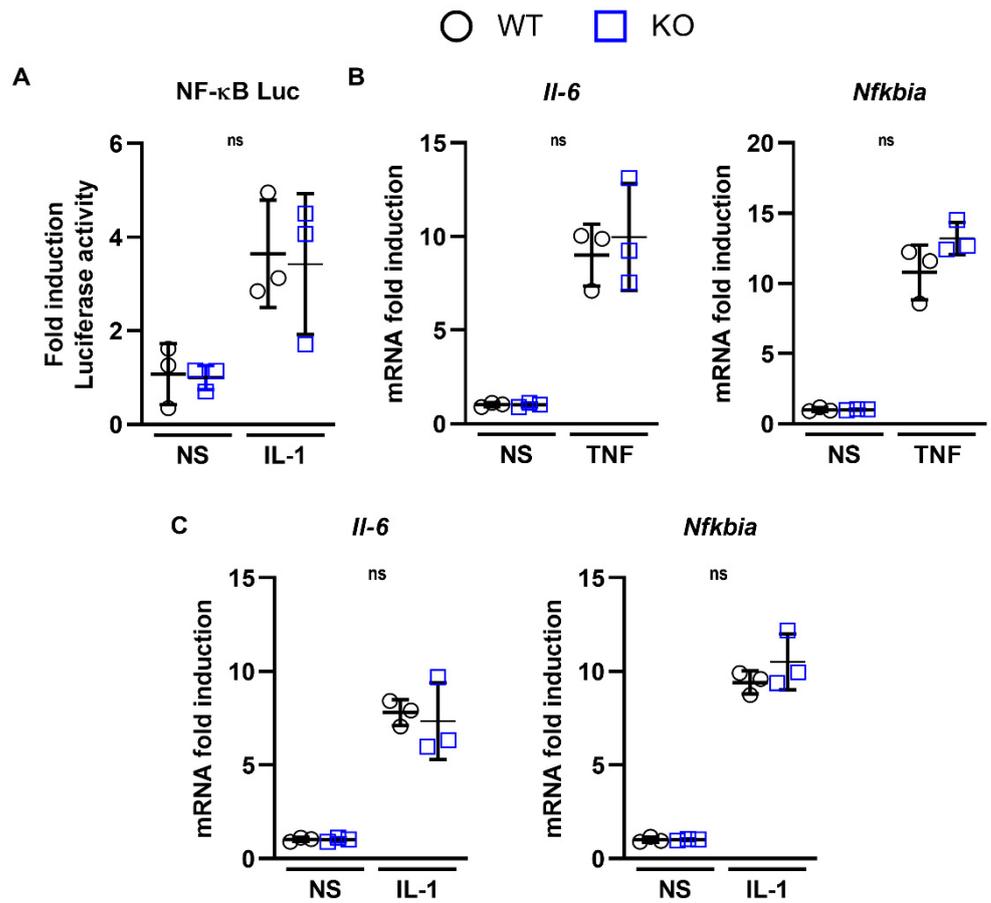


Figure S3. DDX50 (RH-II/Gu β) is not required for NF- κ B-dependent gene transcription. **(A)** WT or *Ddx50*^{-/-} MEFs were transfected with pNF- κ B-Luc or pTK-RL, as an internal control. Cells were left untreated or stimulated for 7 h with 100 ng/ml IL-1 α and Firefly Luciferase activity was measured. **(B and C)** WT or *Ddx50*^{-/-} MEFs were left untreated or stimulated for 1 h with 100 ng/ml TNF α **(B)** or IL-1 α **(C)**. Following mRNA extraction, the fold induction of *Nfkb1a* and *Il-6* mRNA levels relative to *Gapdh* were analysed by RT-qPCR. Representative of 3 independent experiments. For all panels statistical significance was determined by performing a Two-way ANOVA test followed by Tukey's multiple comparison post-hoc test. ns, non-significant.

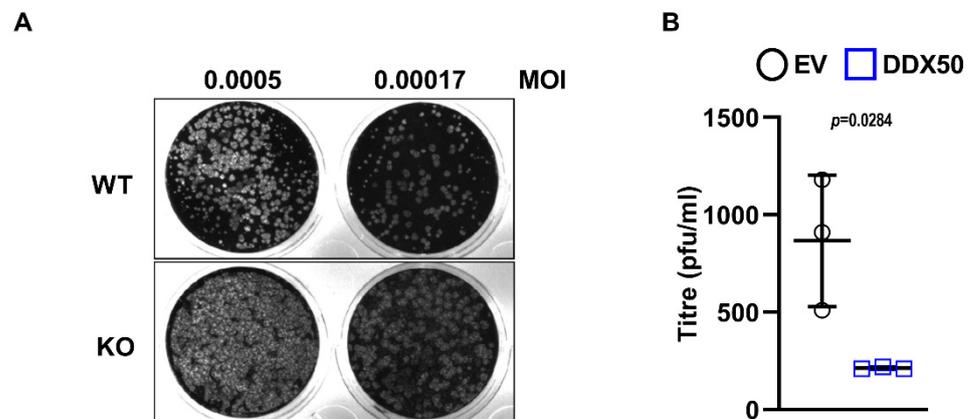


Figure S4. Overexpression of DDX50 inhibits VACV dissemination and replication. **(A)** WT or *Ddx50*^{-/-} MEFs were infected with A5-GFP VACV WR at MOI = 0.0005 or 0.00017 and representative images for enumeration of plaque formation efficiency were taken 24 h p.i. **(B)** HF cells transduced with EV or to express HA tagged DDX28 or DDX50 were infected with 300 p.f.u. VACV and titre

was calculated 48 h p.i. Representative of three independent experiments. Statistical significance was determined by performing a two-tailed unpaired *t*-test.