

Supplementary materials

# DDX50 Is a Viral Restriction Factor That Enhances IRF3 Activation

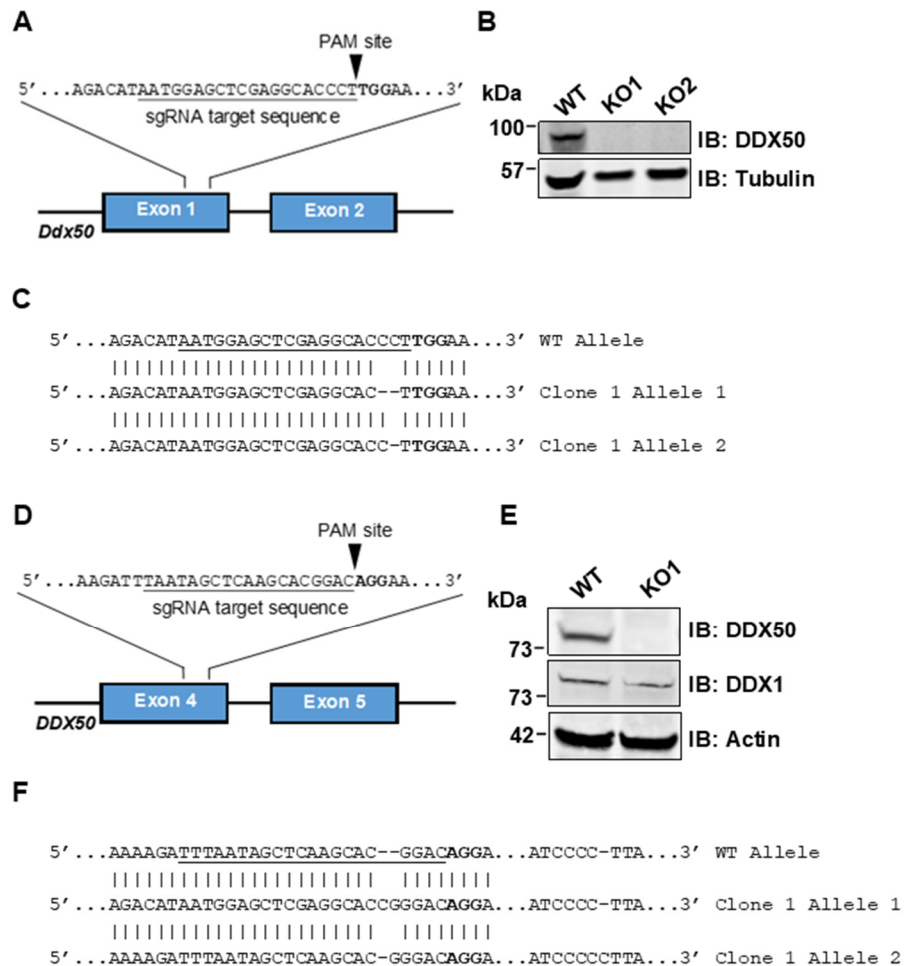
**Table S1.** Constructs and primers used in the study.

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 <sup>r</sup> and Puro <sup>r</sup>	Addgene, 71783
	Primer	RE
pCW57-GFP-P2A- <i>Ddx50</i> -HA	CGACGCGTATGCCCCGGGAACTCCTCTGG GCAGGATCCTTAAGCGTAATCTGGAACATCGTAT	MluI BamHI This study
pCMV-PACK	Packaging plasmid for lentivirus production with HIV Gag, Pol, Rev and Tat under the CMV promoter, Amp <sup>r</sup>	Gift, Dr. H. Laman
pCMV-ENV	VSV-G pseudotyped envelope protein under the CMV promoter for lentivirus production, Amp <sup>r</sup>	Gift, Dr. H. Laman
pLDT-TetR	Lentiviral expression plasmid constitutively expressing the Tetracycline promoter repressor, Amp <sup>r</sup> and Neo <sup>r</sup>	(Everett et al, 2013)
pLDT-MCS	Lentiviral expression plasmid with expression under the control of a Tetracycline inducible CMV promoter, Amp <sup>r</sup> and Puro <sup>r</sup>	(Everett et al, 2013)
	Primer	RE
pLDT- <i>DDX50</i> -HA	CGGCTAGCATGCCTGGGAACTCCTCTGG CGGATATCTTAAGCGTAATCTGGAACATCGTATGGGTAG- TCAAACTCCGTT TGTGGCC	NheI EcoRV This study
pLDT- <i>DDX28</i> -HA	CGGATATCATGGCTCTAACGCGGCCCGGT CGGATATCTTAAGCGTAATCTGGAACATCGTATGGGTAGGTTGCTT- GGGGCA AAGGCTC	EcoRV EcoRI This study
pLDT- <i>Ddx50</i>	CGGCTAGCATGCCCCGGGAACTCCTCTGG CGGATATCTCAGTCAAAATTCCGTTTATG	NheI EcoRV This study
pCDNA4/TO-nTAP	Mammalian expression plasmid with expression under the control of a Tetracycline inducible CMV promoter, Amp <sup>r</sup> and Zeocin <sup>r</sup>	(Pallett et al, 2019)
pMX-CMV-YFP	Retroviral plasmid for viral package signal, transcription and processing, YFP expression and puromycin selectable, Amp <sup>r</sup>	Invitrogen
pMX-CMV-YFP-miR30E <i>DDX1</i> clone 1	tcgagaaggtatattgctgttgacagtgagcgccgggcaatcaaggaacataaatagtgagccacagat cttgaagtccgaggcagtaggcatccgggcaatcaaggaacataatacatctgtggcttcactattatgt	This study
pMX-CMV-YFP-miR30E <i>DDX1</i> clone 2	tcgagaaggtatattgctgttgacagtgagcgcatgtggtctgaagctattaatagtgagccacagat cttgaagtccgaggcagtaggcaagatgtggtctgaagctattaatacatctgtggcttcactattaata	This study
pMX-CMV-YFP-miR30E <i>LacZ</i>	tcgagaaggtatattgctgttgacagtgagcgACGTCGTATTACAACGTCGTGAtagtgaa- gccacagat cttgaagtccgaggcagtaggcaCCGTCGTATTACAACGTCGTGAtacatctgtggcttcac- taTCACGA	This study

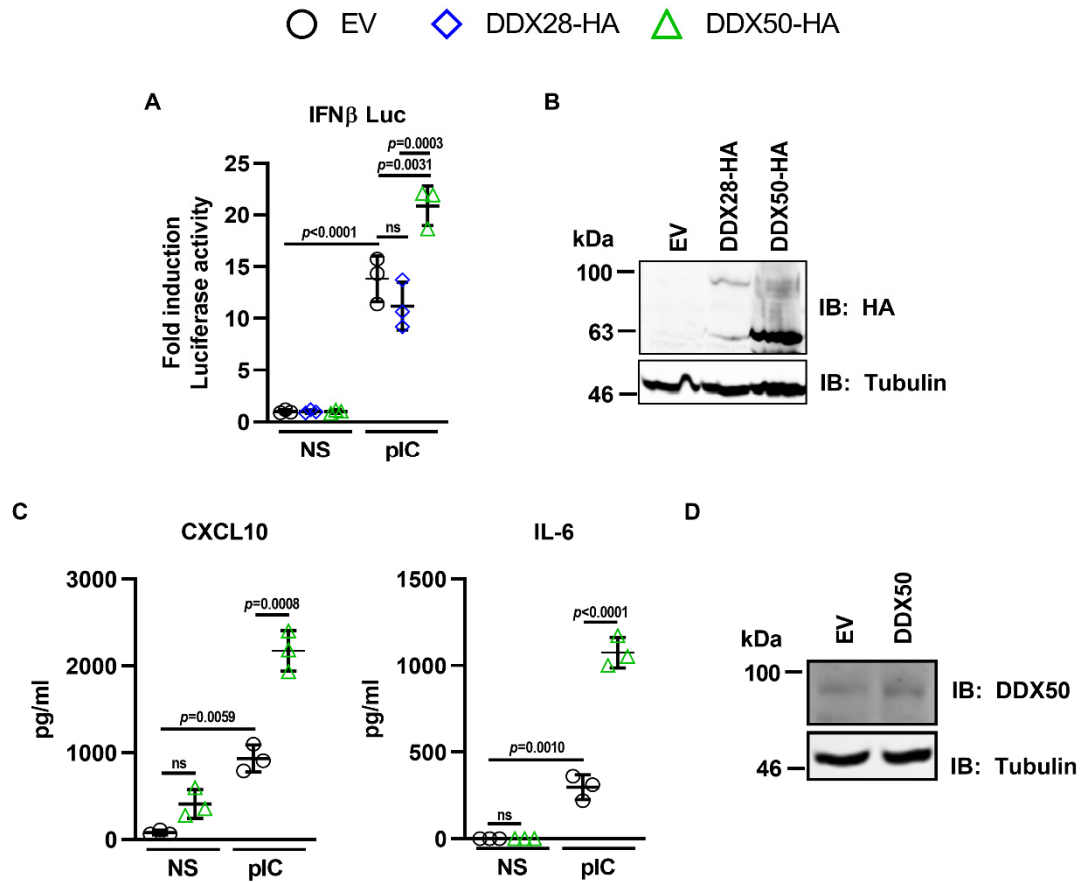
Amp<sup>r</sup>, Ampicillin resistance; Puro<sup>r</sup>, Puromycin resistance; Neo<sup>r</sup>, Neomycin resistance; Zeocin<sup>r</sup>, Zeocin resistance; RE, restriction enzyme.

**Table S2.** Primers for qPCR.

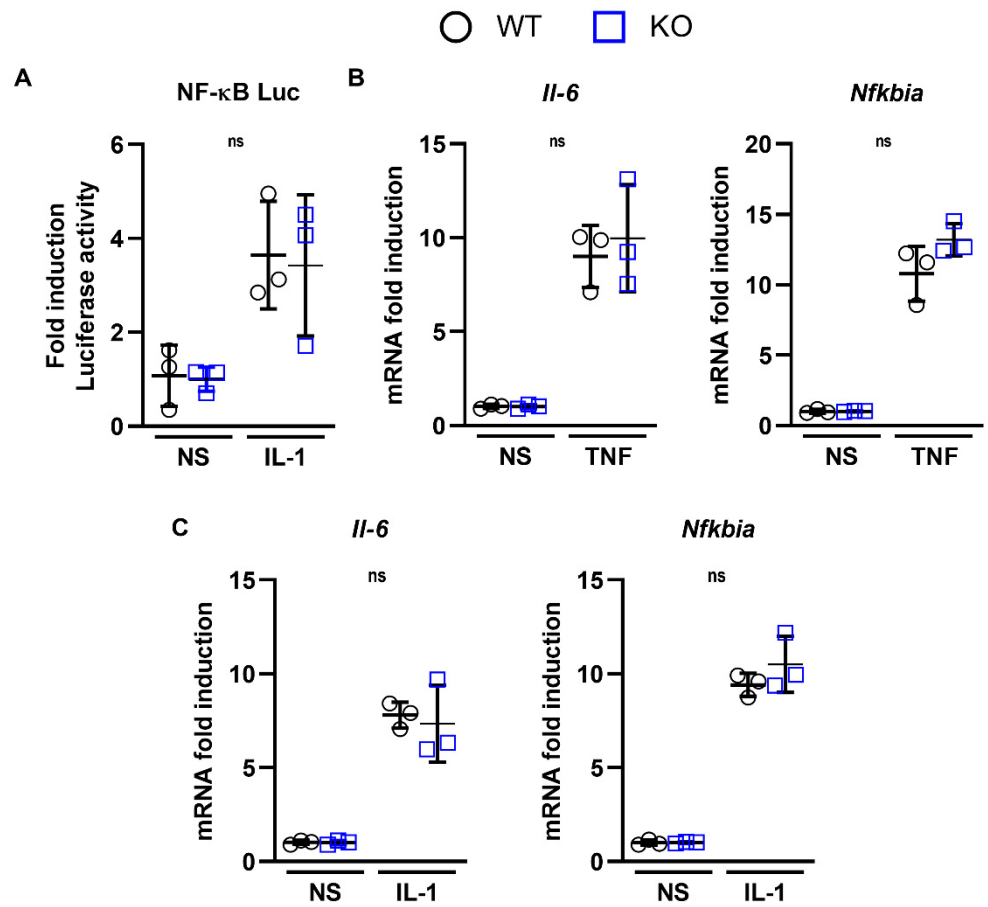
Primer	Sequence
<i>Ifnb</i> Fwd	CATCAACTATAAGCAGCTCCA
<i>Ifnb</i> Rev	TTCAAGTGGAGAGCAGTTGAG
<i>Il-6</i> Fwd	GTAGCTATGGTACTCCAGAAG
<i>Il-6</i> Rev	ACGATGATGCACTTGCAGAA
<i>Cxcl10</i> Fwd	ACTGCATCCATATCGATGAC
<i>Cxcl10</i> Rev	TTCATCGTGGCAATGATCTC
<i>Isg56</i> Fwd	ACCATGGGAGAGAATGCTGAT
<i>Isg56</i> Rev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



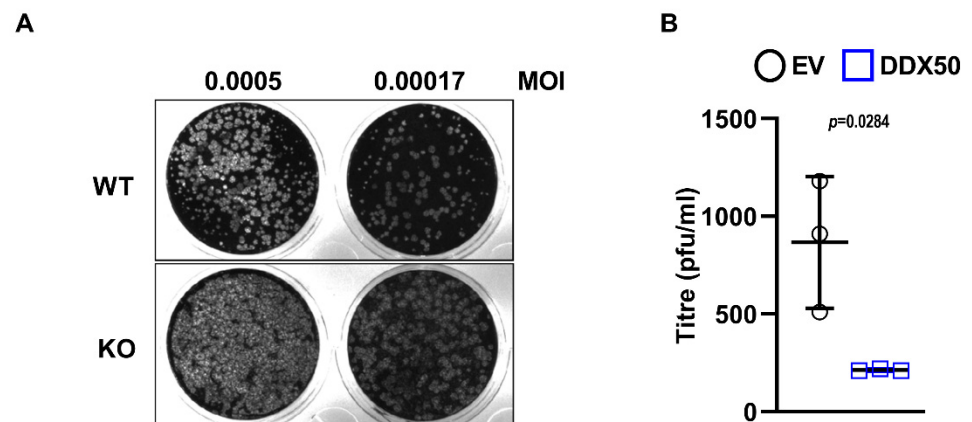
**Figure S1.** CRISPR-Cas9 mediated knockout of *Ddx50/DDX50* (*RH-III/Guβ*). WT mouse (**A-C**) fibroblasts and human HEK293Ts (**D-F**) were transfected with pX459 - sgRNA targeting *Ddx50* exon 1 (**A**) and *DDX50* exon 4 (**D**), respectively. Cells were selected in 4 μg/ml (MEFs) or 1 μg/ml (HEK293T) 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 $\beta$*  promoter or Renilla, and Firefly Luciferase fold induction was analysed following transfection with 5  $\mu$ g/ml Poly IC for 6 h. **(B)** Relative levels of DDX28 and DDX50 expression were confirmed by immunoblotting with anti-HA and anti- $\alpha$ -tubulin. Representative of three independent experiments. **(C)** WT MEF EV or DDX50 transduced cell lines were transfected with lipofectamine only or 5  $\mu$ 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- $\alpha$ -tubulin.



**Figure S3.** DDX50 (RH-II/Guβ) is not required for NF-κB-dependent gene transcription. (A) WT or *Ddx50*<sup>-/-</sup> MEFs were transfected with pNF-κB-Luc or pTK-RL, as an internal control. Cells were left untreated or stimulated for 7 h with 100 ng/ml IL-1α and Firefly Luciferase activity was measured. (B and C) WT or *Ddx50*<sup>-/-</sup> MEFs were left untreated or stimulated for 1 h with 100 ng/ml TNFα (B) or IL-1α (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*<sup>-/-</sup> 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

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