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Supplemental information

Disruption of DNA polymerase ζ engages

an innate immune response

Sara K. Martin, Junya Tomida, and Richard D. Wood



Figure S1. Shortened REV3L construct rescues phenotypes of pol ζ disruption in an additional set of clones, Related to Figure 1

(A) Stable expression of TR4-2 with an N-terminal Flag-HA tag in *Rev3l* KO clones as detected by HA immunoblot. For REVERT total protein loading control see Fig 1 and S1 in Mendeley Data: <u>doi: 10.17632/5348dspthz.1</u>
(B) Stable expression of TR4-2 in *Rev3l* KO MEF clones reverses hypersensitivity to cisplatin. MEFs were exposed to the indicated cisplatin concentrations for 48 hr and relative survival was quantified with the ATPlite assay.







Figure S3. Positive and Negative controls for single-molecule RNA in situ hybridization, Related to Figure 3. (A) Positive Control. Skin section of a keratin 5 conditional heterozygous *Rev3l* mouse (BK.5 Cre *Rev3l*^(+/lox)) was analyzed with RNA in situ hybridization probes specific for the housekeeping genes *Ppib* (blue dots) *and Polr2a* (red dots). Arrows point to one example of probe signals.

(B) Negative control. Single-molecule RNA in situ hybridization in skin of keratin 5 conditional heterozygous *Rev3l* mouse (BK.5 Cre *Rev3l*^(+,/lox)) using a probe specific for the *Bacillus subtillus dapB* gene. As expected, no signal is detected.

	Rev3l KC) + EV vs	<i>Rev3I</i> KO + TR4-2 vs	
	Rev3l H	ET + EV	Rev3l KO + EV	
Gene Name	Log2 Fold Change	FDR	Log2 Fold Change	FDR
Cxcl11	10.08783	1.43E-08	-5.0312	0.000362
Lyz2	8.870484	1.31E-09	-4.772444	1.91E-05
Oas2	8.267013	3.67E-05	-6.567388	0.002339
Oas3	7.959514	3.31E-06	-6.542195	0.000362
lfi44	7.479926	1.44E-06	-4.825919	0.003736
Usp18	6.572054	7.54E-07	-4.059615	0.005592
Rsad2	6.196355	3.40E-05	-4.282616	0.01059
Oas1g	6.161099	0.000707	-5.145596	0.011973
lrf7	5.839463	5.85E-11	-3.041723	0.001875
Mx2	4.957664	0.000188	-3.258566	0.041769
Oas1a	4.870041	0.000783	-4.308707	0.009657
Oasl2	4.500346	9.79E-08	-2.551515	0.010097
Dhx58	4.068542	2.92E-09	-2.264432	0.004736
Cc/20	4.171942	0.004204	-4.143873	0.013561
lfit3	3.995032	9.81E-05	-2.691874	0.032516
lfi27	3.896872	1.04E-07	-1.961613	0.035
lsg15	3.587676	2.05E-07	-2.203871	0.007627
Ccl5	3.546222	3.94E-06	-2.176328	0.021028
lrf5	3.182247	5.70E-05	-0.922754	0.53922
lfih1	3.155781	1.23E-06	-1.783102	0.032395
Oasl1	3.143506	0.000161	-1.113838	0.441074
lfit1	3.131239	6.84E-05	-1.706951	0.113497
Cxcl10	3.042178	8.26E-06	-0.810127	0.544102
Ddx58	2.516276	2.12E-07	-1.110754	0.114366

 Table S1. Log2 Fold Change of 24 selected interferon stimulated genes, Related to Figure 2

Species	Target	Direction	Sequence (5' to 3')	Source
Mouse	Hprt	Forward	CTGGTGAAAAGGACCTCTCG	Mackenzie et al., 2016
Mouse	Hprt	Reverse	CAAGGGCATATCCAACAACA	Mackenzie et al., 2016
Mouse	Ccl5	Forward	ACGTCAAGGAGTATTTCTACAC	Mackenzie et al., 2016
Mouse	Ccl5	Reverse	GATGTATTCTTGAACCCACT	Mackenzie et al., 2016
Mouse	Cxcl11	Forward	AGGAAGGTCACAGCCATAGC	Yang et al., 2007
Mouse	Cxcl11	Reverse	CGATCTCTGCCATTTTGACG	Yang et al., 2007
Mouse	Rsad2	Forward	ATAGTGAGCAATGGCAGCCT	West et al., 2015
Mouse	Rsad2	Reverse	AACCTGCTCATCGAAGCTGT	West et al., 2015
Mouse	Ifih1	Forward	CGGAAGTTGGAGTCAAAGC	West et al., 2015
Mouse	Ifih1	Reverse	TTTGTTCAGTCTGAGTCATGG	West et al., 2015
Mouse	Isg15	Forward	CTAGAGCTAGAGCCTGCAG	West et al., 2015
Mouse	Isg15	Reverse	AGTTAGTCACGGACACCAG	West et al., 2015
Mouse	Irf7	Forward	CAATTCAGGGGATCCAGTTG	West et al., 2015
Mouse	Irf7	Reverse	AGCATTGCTGAGGCTCACTT	West et al., 2015

 Table S2. Primers used for qPCR in this study, Related to STAR Methods.