

	BMDMs			BMDCs		
Mouse Strain	STING	DDX41	cGAS	STING	DDX41	cGAS
C57BL/6	1.243 ± .435	0.731 ± .363	0.654 ± .114	0.666 ± .476	0.530 ± .209	0.170± .02
STING <sup>gt/gt</sup>	0.006 ± .004	0.495 ± .316	0.368 ± .132	0.014 ± .021	0.335 ± .164	0.214 ± .128
cGAS KO	1.294 ± .192	0.670 ± .260	0.001 ± .001	0.65 ± .505	1.10 ± .139*	0.003 ± .003
CD11cCre DDX41 <sup>fl/fl</sup>	1.378 ± .778	0.537 ± .267	0.834 ± .661	1.00±.370	0	0.278±.075
LyCre DDX41 <sup>fl/fl</sup>	1.237 ± .486	0.003 ± .005	1.407 ± .744	0.89 ± .332	0.469±.172	0.29± .11

	IFNβ/actin (x10 <sup>-3</sup> )			
Mouse	BMDM	BMDC		
C57BL/6	0.017 ± 0.002	1.1 ± 0.7		
STING <sup>gt/gt</sup>	0.015 ± 0.021	ND		
cGAS KO	0.016 ± 0.003	0.8 ± 0. 1		
LyCre DDX41 <sup>fl/fl</sup>	0.022 ± 0.003	ND		
CD11cre DDX41 <sup>fl/fl</sup>	ND	0.9±0.6		

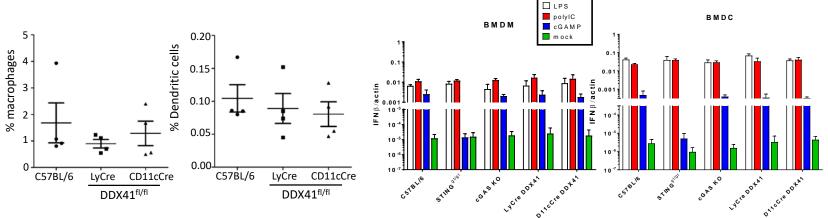


FIG S4 Characterization of Ddx41 KO mice. Related to Fig. 5 and 6. (A) Mapof the Ddx41 locus and inserted loxP sites. Expression of Cre recombinase results in the deletion of exons 7 to 9. (B) Quantification of DDX41, cGAS, and STING protein in various knockout mouse cells. Shown are the means ± SDs for 3 independent Western blotting assays of cells from 3 different mice of each strain. \*,  $P \le 0.05$  compared to BL/6,  $Sting^{gt/gt}$ , and CD11cCreDDX41 (unpaired t test). (C) Basal IFN-β RNA levels in Ddx41 KO BMDMs and BMDCs. RNA was isolated from BMDMS of 3 mice and BMDCs of 2 mice each of the indicated genotypes, and qPCR was performed for IFN-β levels, using a standard curve to measure relative levels. Shown are the means ± SDs. Abbreviations: ND, not done. (D) PBMCs from 4 mice of each genotype were stained with conjugated anti-CD11c (DC) or anti-F4/80 (macrophage) antibodies and analyzed by FACS. (E) Treatment of Ddx41 KO BMDMs and BMDCs with different ligands. BMDMs and BMDCs from the cGas, LyCre DDX41 and CD11cCre-DDX41 KO and  $Sting^{gt/gt}$  mice were treated with the indicated ligands, as described in Materials and Methods. RNA was isolated after 6 h of treatment for all ligands and subjected to RT-qPCR. Shown are the averages from 2 experiments (triplicate technical replicates) with cells isolated from different mice.