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Supplementary Table 1. Identification of a heterozygous WWP2 missense mutation

Inheritance	Zygoty	Gene and attributes					Variants and attributes							Remarks
		Gene	DOMINO	KO mice (MGI)	Disease associated (OMIM)	Link with TLR3	Change	Frequency (gnomAD)	Origin	ClinVar	Amino-acid	MutScore	CADD	
Recessive	Homozygous	None												
	Compound heterozygous	ZFYVE26	Not relevant for recessive inheritance	No immunological phenotypes	Spastic paraplegia autosomal recessive	No	c.3881A>G	NA	Mother	No	Not conserved	0.053	15.9	Not retained: mother variant with low deleteriousness prediction scores; association with an another disease and no link with immunological phenotype or function.
							c.961A>C	0.0134%	Father	Yes (VUS)	Conserved	0.383	27.7	
		FLNB	Not relevant for recessive inheritance	Immune phenotype	Larsen syndrome, autosomal dominant; Atelosteogenesis, autosomal dominant	No	c.2237A>C	0.0012%	Mother	No	Conserved	0.800	26.5	Not retained: association with other unrelated diseases
							c.4186G>A	0.0028%	Father	No	Conserved	0.260	23.0	
c.5327T>C	0.0028%	Father	Yes (VUS)	Conserved	0.864	28.4								
Dominant	De novo	RAB13	<0.9	No immunological phenotypes	NA	No	c.429T>G	0.0004%	De novo	No	Conserved	0.342	3.6	Not retained: DOMINO score lower than 0.9 suggesting the absence of an association with a dominant phenotype and no link with immunological phenotypes
		CNTNAP2	<0.9	No immunological phenotypes	NA	No	c.3427G>C	0.0032%	De novo	No	Conserved	NA	22.9	Not retained: DOMINO score lower than 0.9 suggesting the absence of an association with a dominant phenotype and no link with immunological phenotypes
	Heterozygous	VANGL2	0.994	No immunological phenotypes	Neural tube defects, autosomal dominant	No	c.931G>A	0.0018%	Mother	No	Conserved	0.609	31.0	Not retained: association with an another disease and no link with immunological phenotype or function.
		WWP2	0.987	Immune phenotype	NA	Yes	c.2522G>A	0.0012%	Mother	No	Conserved	0.933	30.0	Retained variant

VUS: variant of uncertain significance; red means attribute pointing towards benign; green means attribute pointing towards pathogenic.

Supplementary Table 2. Experimental structures fetched and used for the $\Delta\Delta G$ calculations.

Protein	PDB ID	Position of the Arg
ITCH	5c7m[11]	874
ITCH	4be8[12]	874
NEDD4	2xbb[13]	870
NEDD4	5c7j[11]	870
NEDD4	2xbf[13]	870
NEDD4	4bbn[12]	870
NEDD4	5c7j[11]	870
NEDD4	5c91[14]	870
NEDD4L	3jw0[15]	925
NEDD4L	3jvz[15]	925
NEDD4L	2oni	925
NEDD4L	5hpk[11]	925
NEDD4L	3tug	925
SMURF2	1zvd[16]	719
WWP1	5hpt[11]	893
WWP1	1nd7[17]	893
WWP1	5hps[11]	893
WWP1	6j1y[10]	893
WWP2	4y07[7]	841
WWP2	5tj7[9]	841
WWP2	5tj8[9]	841
WWP2	5tjq[9]	841
WWP2	6j1z[10]	841

Supplementary Table 3. Alignment metrics

METRIC	Medium					
	M1	M2	M3	M4	M5	M6
	WWP2 p.R841H			WWP2 p.R841		
TOTAL_READS	61404314	56700356	49370062	45803731	39504625	51486150
QUALITY_TRIMMED_BASES_CUTADAPT	3108195	2832236	2412693	2208621	1954858	2585471
DISCARDED_RRNA	1324677	1242723	1118972	874830	796844	961455
DISCARDED_LOW_COMPLEXITY	2015832	1904131	1562551	1420469	1182211	1519207
CLEAN_READS	58063805	53553502	46688539	43508432	37525570	49005488
CLEAN_READS_ALIGNED	57630213	53156202	46298847	43174694	37224466	48525756
PCT_CLEAN_READS_ALIGNED	99.25	99.26	99.17	99.23	99.20	99.02
CLEAN_ALIGNED_BASES	5714999190	5271242811	4584518715	4280282645	3684401479	4798146339
MEAN_READ_LENGTH	100.63	100.63	100.63	100.63	100.63	100.63
READS_ALIGNED_IN_PAIRS	0	0	0	0	0	0
PCT_READS_ALIGNED_IN_PAIRS	0	0	0	0	0	0
STRAND_BALANCE	0.49	0.49	0.49	0.49	0.49	0.49
UNMAPPED_READS	433592	397300	389692	333738	301104	479732
UNPAIRED_READ_DUPLICATES	45560639	41166756	36076528	33046874	28560896	37173925
READ_PAIR_OPTICAL_DUPLICATES	0	0	0	0	0	0
UNIQUELY_ALIGNED	53510871	49385004	42771873	39910933	34156643	45031471
MULTIPLE_ALIGNMENT	4119342	3771198	3526974	3263761	3067823	3494285
READ_ALIGNED_TO_REF_EXONS	49788822	45731048	39749318	37099139	31733428	41742606
NO_FEATURE	1720314	1824510	1347082	1239129	999908	1564898
AMBIGUOUS	2001735	1829446	1675473	1572665	1423307	1723967
TOO_LOW_AQUAL	0	0	0	0	0	0
NOT_ALIGNED	433592	397300	389692	333738	301104	479732
ALIGNMENT_NOT_UNIQUE	10518228	9685821	9010348	8362435	7952180	8953669
BIAS_INDEX_50	54.11	54.01	56.14	55.30	57.89	54.46
SOVER3_COV_RATIO	0.72	0.73	0.62	0.66	0.55	0.71
UNMAPPED_READS_ALIGNED_TO_MITOCHONDRIA	3659	3042	3911	3564	3849	3838
UNMAPPED_READS_ALIGNED_TO_MITOCHONDRIA_%	0.84	0.77	1.00	1.07	1.28	0.80
MAPPED_READS_ALIGNED_TO_MITOCHONDRIA	851676	732384	813191	819909	728887	631739
MAPPED_READS_ALIGNED_TO_MITOCHONDRIA_%	1.48	1.38	1.76	1.90	1.96	1.30

Supplementary Table 3 continued.

METRIC	3h Poly(I:C) stimulation					
	P1	P2	P3	P4	P5	P6
	WWP2 p.R841H			WWP2 p.R841		
TOTAL_READS	48728829	47270714	43680905	52993268	39831724	41632801
QUALITY_TRIMMED_BASES_CUTADAPT	2428027	2501289	2373526	2667698	2128498	2081268
DISCARDED_RRNA	1060753	1072967	1240241	1247690	885686	879336
DISCARDED_LOW_COMPLEXITY	1436123	1517397	1447652	1751882	1291267	1325801
CLEAN_READS	46231953	44680350	40993012	49993696	37654771	39427664
CLEAN_READS_ALIGNED	45783710	44182694	40427824	49563315	37319708	39121426
PCT_CLEAN_READS_ALIGNED	99.03	98.89	98.62	99.14	99.11	99.22
CLEAN_ALIGNED_BASES	4529804390	4375223110	4003958595	4909172403	3698618521	3880188262
MEAN_READ_LENGTH	100.62	100.59	100.62	100.63	100.62	100.63
READS_ALIGNED_IN_PAIRS	0	0	0	0	0	0
PCT_READS_ALIGNED_IN_PAIRS	0	0	0	0	0	0
STRAND_BALANCE	0.48	0.48	0.48	0.48	0.48	0.48
UNMAPPED_READS	448243	497656	565188	430381	335063	306238
UNPAIRED_READ_DUPLICATES	34448409	33172503	29731483	37897026	27149076	28731985
READ_PAIR_OPTICAL_DUPLICATES	0	0	0	0	0	0
UNIQUELY_ALIGNED	42499812	40931521	37526946	45977698	34615723	36306352
MULTIPLE_ALIGNMENT	3283898	3251173	2900878	3585617	2703985	2815074
READ_ALIGNED_TO_REF_EXONS	39041026	37738884	34506696	42144811	31806447	33421944
NO_FEATURE	1892503	1665129	1631048	2109850	1511784	1528196
AMBIGUOUS	1566283	1527508	1389202	1723037	1297492	1356212
TOO_LOW_AQUAL	0	0	0	0	0	0
NOT_ALIGNED	448243	497656	565188	430381	335063	306238
ALIGNMENT_NOT_UNIQUE	8358927	8273480	7343938	9114837	6871505	7136669
BIAS_INDEX_50	53.24	53.25	53.13	52.96	53.09	53.24
SOVER3_COV_RATIO	0.78	0.77	0.78	0.79	0.78	0.77
UNMAPPED_READS_ALIGNED_TO_MITOCHONDRIA	8744	8650	11398	7827	6745	4984
UNMAPPED_READS_ALIGNED_TO_MITOCHONDRIA_%	1.95	1.74	2.02	1.82	2.01	1.63
MAPPED_READS_ALIGNED_TO_MITOCHONDRIA	1631704	1616308	1507569	1776657	1474545	1319356
MAPPED_READS_ALIGNED_TO_MITOCHONDRIA_%	3.56	3.66	3.73	3.58	3.95	3.37

Supplementary Table 4. AP-1 and NF-kB downstream enrichment for the difference in Poly(I:C) stimulation between WWP2 p.R841H and WWP2 p.R841.

Pathway	FDR adjusted-p value	Normalized Enrichment Score
NF-kB atypical	4.02e-01	1.09
NF-kB non-canonical	4.02e-01	-1.10
NF-kB canonical	3.02e-02	-1.65
TNF: AP-1 Downstream	3.55e-05	-2.07

Complete pathways	FDR adjusted-p value	Normalized Enrichment Score
hsa:04064	7.32e-02	-1.37
hsa:04668	7.89e-05	-1.90

P-values were computed using the adaptive multi-level split Monte-Carlo approach of R package fgsea. Positive and negative enrichment are considered (two-sided). P-values were corrected for multiple testing using False Discovery Rate. FDR stands for False Discovery Rate.

Supplementary Table 5. Primers.

	Forward	Reverse
TLR3	5'-GTGCCAGAACTTCCCATGT-3'	5'-TCCAGCTGAACCTGAGTTCC-3'
WWP2	5'-CTTGTCGTGTCGTTCCGCTT-3'	5'-ACCATTGCTTCACACTTGACTT-3'
TRIF	5'-GCCAGCAACTTGGAAATCAGC-3'	5'-GGGGTCGTCACAGAGCTTG-3'
IFNB	5'-CATTACCTGAAGGCCAAGGA-3'	5'-CAGCATCTGCTGGTTGAAGA-3'
ISG56	5'-CCTCCTTGGGTTCTGTCTACA-3'	5'-GGCTGATATCTGGGTGCCTA-3'
MX1	5'-ACCTACAGCTGGCTCCTGAA-3'	5'-CGGCTAACGGATAAGCAGAG-3'
IFIT2	5'-ACAAGGCCATCCACCACTTTAT-3'	5'-CCCAGCAATTCAGGTGTTAACA-3'
TNF	5'-CAGAGGGCCTGTACCTCATC-3'	5'-GGAAGACCCCTCCAGATAG-3'
HPRT	5'-GAACGTCTTGCTCGAGATGTG-3'	5'-CCAGCAGGTCAGCAAAGAATT-3'

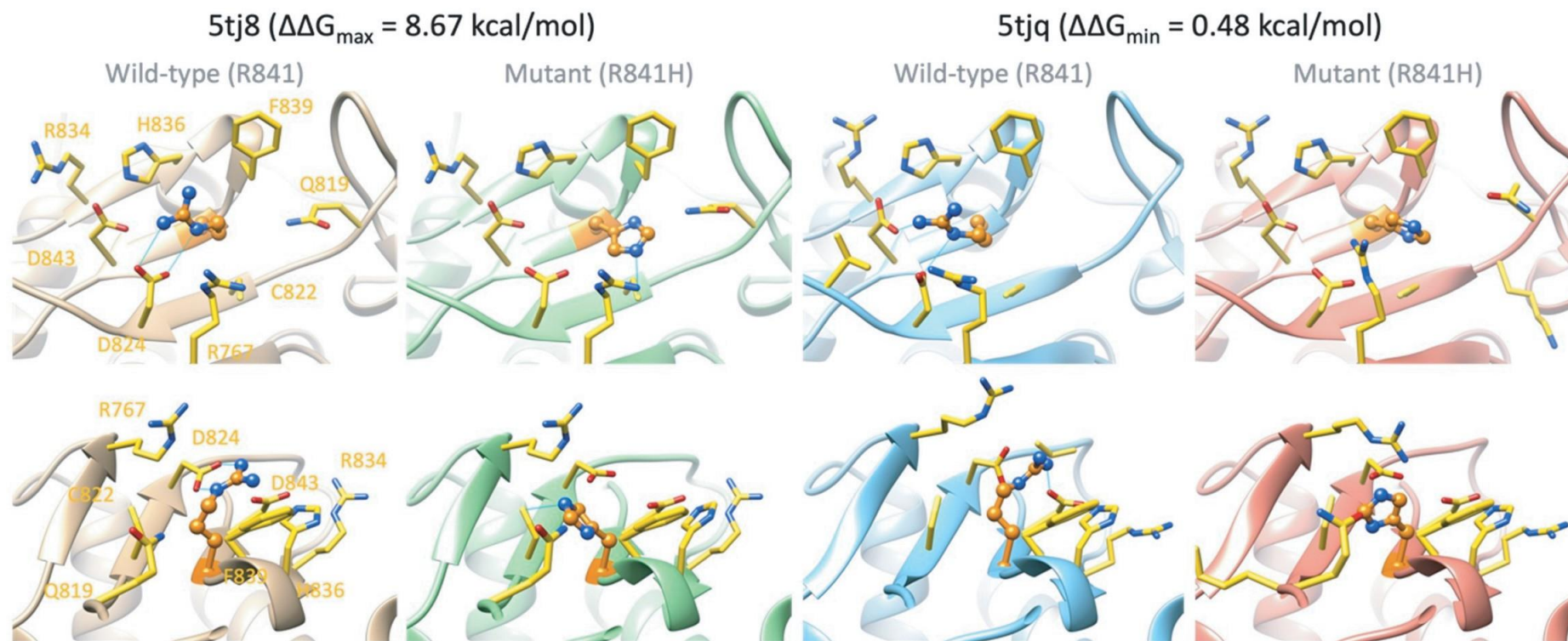
Supplementary Figure 1. Conservation of the Arginine 841 in vertebrate WWP2 orthologues

Organism	Begin	Amino acids (green = different from human)																												End																		
H.sapiens	779	F	W	Q	V	V	K	E	M	D	N	E	K	R	I	R	L	L	Q	F	V	T	G	T	C	R	L	P	V	G	G	F	A	E	L	I	G	S	N	G	P	Q	K	F	C	I	D	824
M.mulatta	779	F	W	Q	V	V	K	E	M	D	N	E	K	R	I	R	L	L	Q	F	V	T	G	T	C	R	L	P	V	G	G	F	A	E	L	I	G	S	N	G	P	Q	K	F	C	I	D	824
C.lupus	791	F	W	Q	V	V	K	E	M	D	N	E	K	R	I	R	L	L	Q	F	V	T	G	T	C	R	L	P	V	G	G	F	A	E	L	I	G	S	N	G	P	Q	K	F	C	I	D	836
B.taurus	779	F	W	Q	V	V	K	E	M	D	N	E	K	R	I	R	L	L	Q	F	V	T	G	T	C	R	L	P	V	G	G	F	T	E	L	I	G	S	N	G	P	Q	K	F	C	I	D	824
M.musculus	779	F	W	Q	V	V	K	E	M	D	N	E	K	R	I	R	L	L	Q	F	V	T	G	T	C	R	L	P	V	G	G	F	A	E	L	I	G	S	N	G	P	Q	K	F	C	I	D	824
R.norvegicus	779	F	W	Q	V	V	K	E	M	D	N	E	K	R	I	R	L	L	Q	F	V	T	G	T	C	R	L	P	V	G	G	F	A	E	L	I	G	S	N	G	P	Q	K	F	C	I	D	824
G.gallus	833	F	W	Q	V	V	K	E	M	D	N	E	K	R	I	R	L	L	Q	F	V	T	G	T	C	R	L	P	V	G	G	F	A	E	L	I	G	S	N	G	P	Q	K	F	C	I	D	878
D.rerio	775	F	W	Q	V	V	K	E	M	D	N	E	K	R	I	R	L	L	Q	F	V	T	G	T	C	R	L	P	V	G	G	F	A	E	L	I	G	S	N	G	P	Q	K	F	C	I	D	820
X.tropicalis	638	F	W	Q	V	V	K	E	M	D	N	E	K	R	I	R	L	L	Q	F	V	T	G	T	C	R	L	P	V	G	G	V	E	L	I	G	S	N	G	P	Q	K	F	C	I	D	683	

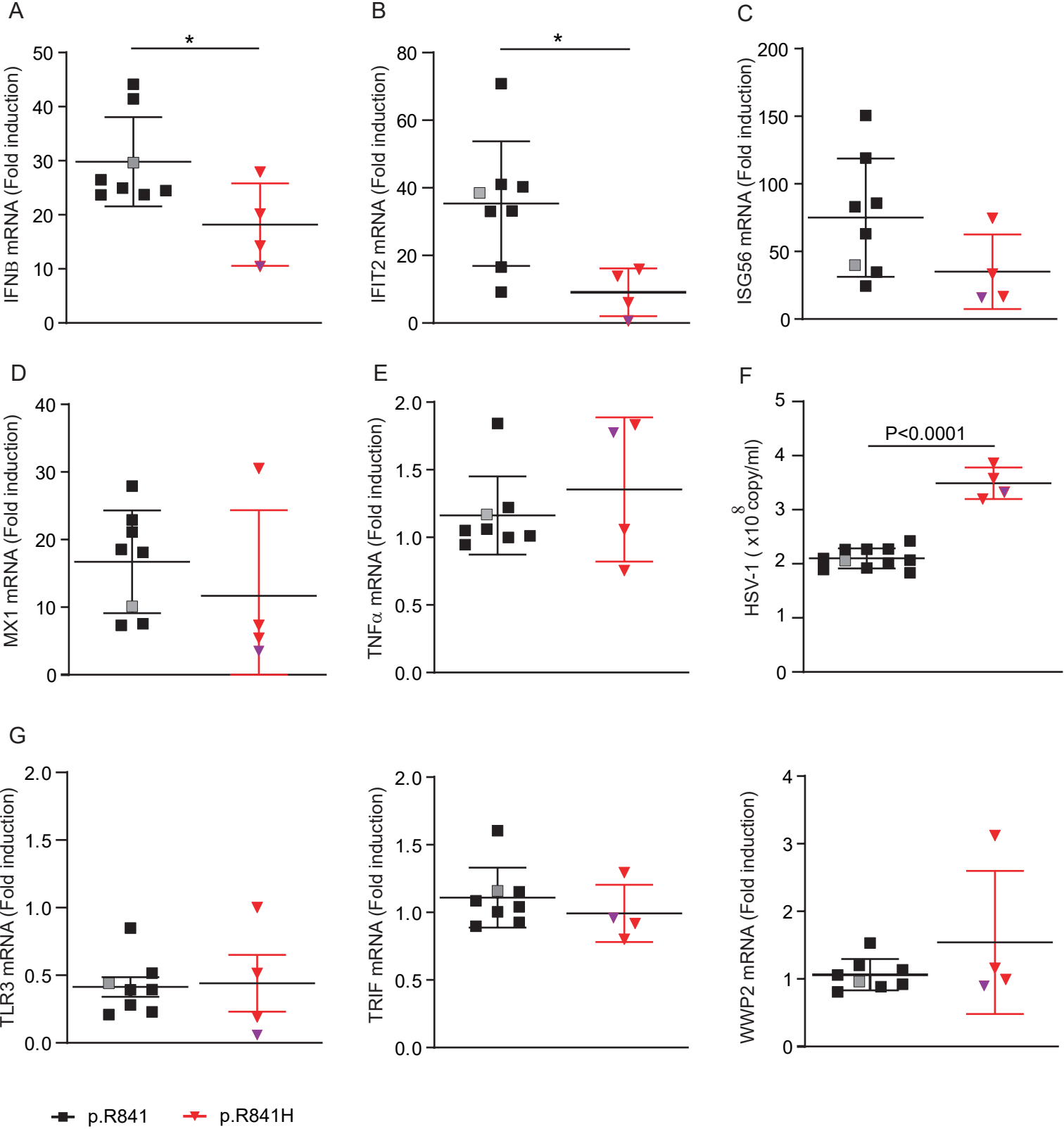
Organism	Begin	Amino acids (green = different from human, red = R841 in human)																												End																		
H.sapiens	825	K	V	G	K	E	T	W	L	P	R	S	H	T	C	F	N	R	L	D	L	P	P	Y	K	S	Y	E	Q	L	R	E	K	L	L	Y	A	I	E	E	T	E	G	F	G	Q	E	870
M.mulatta	825	K	V	G	K	E	T	W	L	P	R	S	H	T	C	F	N	R	L	D	L	P	P	Y	K	S	Y	E	Q	L	R	E	K	L	L	Y	A	I	E	E	T	E	G	F	G	Q	E	870
C.lupus	837	K	V	G	K	E	T	W	L	P	R	S	H	T	C	F	N	R	L	D	L	P	P	Y	K	S	Y	E	Q	L	R	E	K	L	L	Y	A	I	E	E	T	E	G	F	G	Q	E	882
B.taurus	825	K	V	G	K	E	T	W	L	P	R	S	H	T	C	F	N	R	L	D	L	P	P	Y	K	S	Y	E	Q	L	K	E	K	L	L	Y	A	V	E	E	T	E	G	F	G	Q	E	870
M.musculus	825	R	V	G	K	E	T	W	L	P	R	S	H	T	C	F	N	R	L	D	L	P	P	Y	K	S	Y	E	Q	L	K	E	K	L	L	Y	A	I	E	E	T	E	G	F	G	Q	E	870
R.norvegicus	825	R	V	G	K	E	T	W	L	P	R	S	H	T	C	F	N	R	L	D	L	P	P	Y	K	S	Y	E	Q	L	K	E	K	L	L	Y	A	I	E	E	T	E	G	F	G	Q	E	870
G.gallus	879	K	V	G	K	E	T	W	L	P	R	S	H	T	C	F	N	R	L	D	L	P	P	Y	K	S	Y	E	Q	L	K	E	K	L	L	Y	A	I	E	E	T	E	G	F	G	Q	E	924
D.rerio	821	K	V	G	K	E	T	W	L	P	R	S	H	T	C	F	N	R	L	D	L	P	P	Y	K	N	L	E	Q	L	R	E	K	L	L	F	A	I	E	E	T	E	G	F	G	Q	E	866
X.tropicalis	684	R	V	G	K	D	T	W	L	P	R	S	H	T	C	F	N	R	L	D	L	P	P	Y	K	S	Y	E	Q	L	K	E	K	L	L	F	A	I	E	E	T	E	G	F	G	Q	E	729

Organism	Protein Accession	Organism	Protein Accession
H.sapiens	NP_001257383.1	R.norvegicus	NP_001099654.1
M.mulatta	XP_001101869.1	G.gallus	NP_001034349.1
C.lupus	XP_851140.3	D.rerio	NP_001092918.1
B.taurus	NP_001076883.1	X.tropicalis	NP_001090841.1
M.musculus	NP_080106.1		

Supplementary Figure 3. Maximal and Minimal Energetic impact observed by the introduction of a R814H mutation in the HECT domain of WWP2.

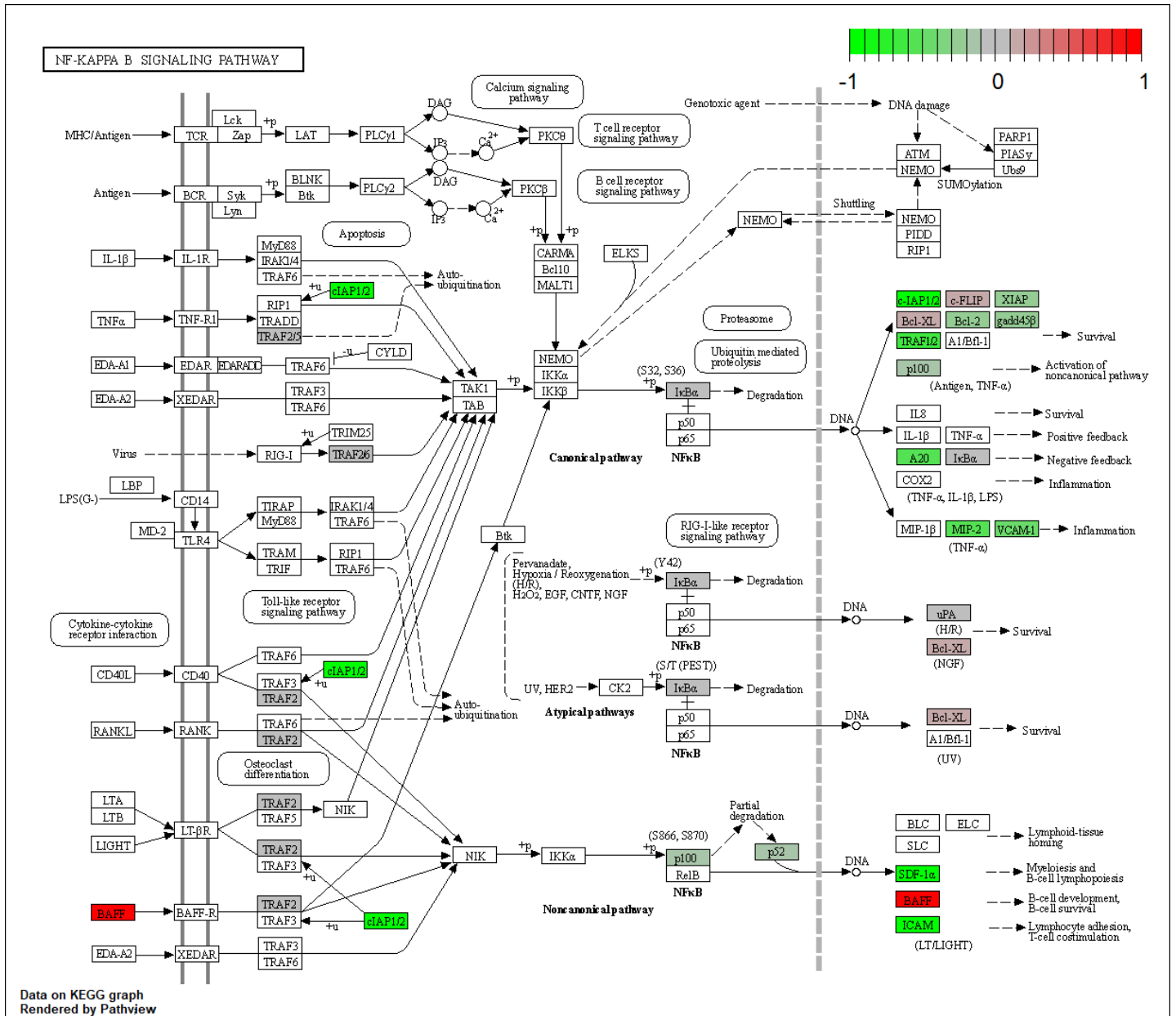


Supplementary Figure 4. WWP2-dependent IFN responses in PBMCs.

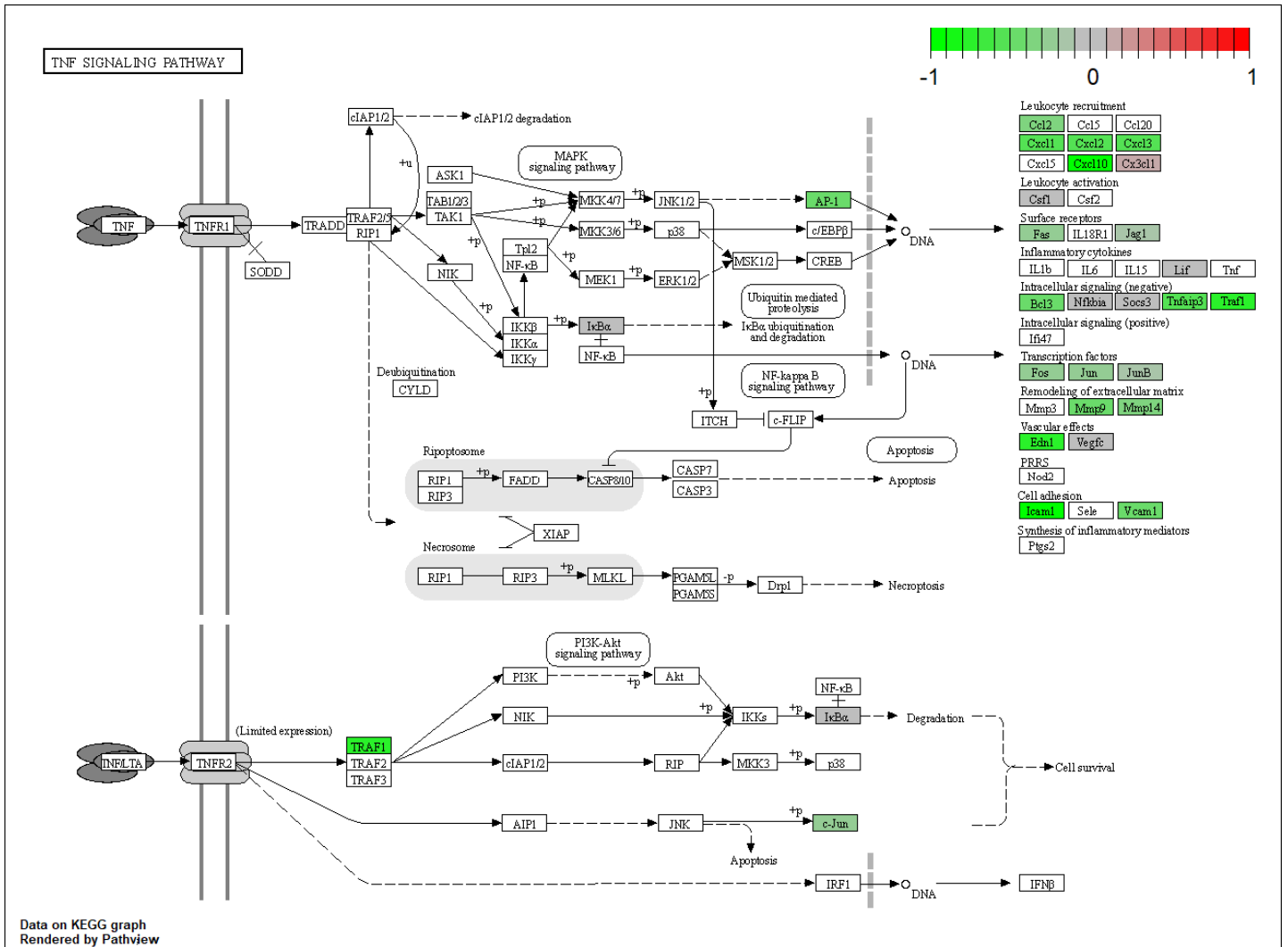


Expression of IFN β (A), IFIT2 (B), ISG56 (C), MX1 (D), TNF α (E) as well as TLR3, TRIF, WWP2 (G) mRNA and quantification of the number of viral genome (F) in PBMCs from individuals carrying p.R841 WWP2 (black, N=8-11) or from individuals carrying p.R841H WWP2 (red, N=4) stimulated with Poly(I:C). Results represent the mean \pm standard error of 1 representative experiment among 4. Statistical analyses were performed using an unpaired two-tailed Student t-test. The purple triangle and the grey square correspond to patient with HSE and to patient's father respectively. * indicates P=0.0405(A) and P=0.02 (B).

Supplementary Figure 5 Significant downregulation of NFKB pathway downstream target genes in WWP2 p.R841H compared to WWP2 p.R841.



Supplementary Figure 6. Significant downregulation of AP-1 downstream target genes in WWP2 p.R841H compared to WWP2 p.R841.



Data on KEGG graph
Rendered by Pathview