

Supplementary Material

		Renilla (RLU)			Firefly (RLU)			Firefly/Renilla (F/R)			Mean (F/R)	%			Mean (%)	S.D. (%)
Experiment 1																
NT	EV	200300	201930	193190	300	340	450	0.0015	0.0017	0.0023	0.0018	2	3	4	3	0.7
	ORF1	-	520410	502360	-	2340	2280	-	0.0045	0.0045	0.0045	-	7	7	7	0.0
	MetYPCP	264980	282670	235400	290	320	190	0.0011	0.0011	0.0008	0.0010	2	2	1	2	0.3
	PCP	177860	200810	186360	220	220	350	0.0012	0.0011	0.0019	0.0014	2	2	3	2	0.7
	Met	136510	171450	148370	120	220	220	0.0009	0.0013	0.0015	0.0012	1	2	2	2	0.5
	MetY	241570	264610	261210	300	420	270	0.0012	0.0016	0.0010	0.0013	2	3	2	2	0.5
	YPCP	187780	170320	223080	370	260	450	0.0020	0.0015	0.0020	0.0018	3	2	3	3	0.4
	Y	324560	433740	414400	550	690	750	0.0017	0.0016	0.0018	0.0017	3	3	3	3	0.2
	X	233610	352240	365320	290	300	350	0.0012	0.0009	0.0010	0.0010	2	1	2	2	0.3
	MV-V	271880	268710	256650	200	300	220	0.0007	0.0011	0.0009	0.0009	1	2	1	1	0.3
IFN	EV	216550	222760	207050	14800	13890	11800	0.0683	0.0624	0.0570	0.0626	109	100	91	100	9.1
	ORF1	558780	579090	550160	24400	25720	22420	0.0437	0.0444	0.0408	0.0429	70	71	65	69	3.1
	MetYPCP	328990	291910	280530	6050	5840	6230	0.0184	0.0200	0.0222	0.0202	29	32	35	32	3.1
	PCP	214980	231230	233310	8470	9380	8770	0.0394	0.0406	0.0376	0.0392	63	65	60	63	2.4
	Met	209910	201380	191460	15920	14320	13570	0.0758	0.0711	0.0709	0.0726	121	114	113	116	4.5
	MetY	307740	297750	333400	14070	12090	14430	0.0457	0.0406	0.0433	0.0432	73	65	69	69	4.1
	YPCP	245040	252760	215480	15830	15870	14360	0.0646	0.0628	0.0668	0.0647	103	100	107	103	3.1
	Y	485760	520570	427280	25220	24900	19510	0.0519	0.0478	0.0457	0.0485	83	76	73	77	5.1
	X	442440	417760	424150	14950	17650	19960	0.0338	0.0422	0.0471	0.0410	54	68	75	66	10.7
	MV-V	314250	303040	312720	740	930	760	0.0024	0.0031	0.0024	0.0026	4	5	4	4	0.6
Experiment 2																
NT	EV	70170	66930	65930	130	80	150	0.00185	0.0012	0.00228	0.0018	9	6	11	9	2.7
	ORF1	149490	167270	167870	110	140	100	0.00074	0.00084	0.0006	0.0007	4	4	3	4	0.6
	MetYPCP	36800	29700	44740	60	60	60	0.00163	0.00202	0.00134	0.0017	8	10	7	8	1.7
	PCP	48340	43100	50030	40	90	20	0.00083	0.00209	0.0004	0.0011	4	10	2	5	4.3
	Met	29500	28030	30250	40	50	70	0.00136	0.00178	0.00231	0.0018	7	9	11	9	2.4
	MetY	46100	44970	50250	110	60	130	0.00239	0.00133	0.00259	0.0021	12	7	13	10	3.3
	YPCP	65090	81310	79390	60	110	140	0.00092	0.00135	0.00176	0.0013	5	7	9	7	2.1
	Y	55110	42850	46580	130	30	60	0.00236	0.0007	0.00129	0.0014	12	3	6	7	4.2
	X	112680	127890	119500	120	130	70	0.00106	0.00102	0.00059	0.0009	5	5	3	4	1.3
	MV-V	35540	40000	30880	90	100	20	0.00253	0.0025	0.00065	0.0019	13	12	3	9	5.3
IFN	EV	81100	90050	87250	1540	1610	2080	0.01899	0.01788	0.02384	0.02024	94	88	118	100	15.7
	ORF1	226430	222010	192670	2450	3660	2780	0.01082	0.01649	0.01443	0.01391	53	81	71	69	14.2
	MetYPCP	51430	60800	52650	790	800	740	0.01536	0.01316	0.01406	0.01419	76	65	69	70	5.5
	PCP	46860	43900	46970	970	1190	1270	0.0207	0.02711	0.02704	0.02495	102	134	134	123	18.2
	Met	37880	49790	45390	1180	1190	1190	0.03115	0.0239	0.02622	0.02709	154	118	130	134	18.3
	MetY	54120	57500	65730	1850	2100	2540	0.03418	0.03652	0.03864	0.03645	169	180	191	180	11.0
	YPCP	92860	93870	77410	2280	2420	1860	0.02455	0.02578	0.02403	0.02479	121	127	119	122	4.4
	Y	84040	81300	77870	1870	1660	1840	0.02225	0.02042	0.02363	0.02210	110	101	117	109	8.0
	X	183290	152810	116600	3450	2990	2390	0.01882	0.01957	0.0205	0.01963	93	97	101	97	4.1
	MV-V	49480	75870	57270	100	130	60	0.00202	0.00171	0.00105	0.00159	10	8	5	8	2.5
Experiment 3																
NT	EV	202890	217220	173590	250	450	250	0.00123	0.00207	0.00144	0.00158	4	6	4	5	1.4
	ORF1	329300	344920	331430	1430	1500	1200	0.00434	0.00435	0.00362	0.00410	13	13	11	13	1.3
	MetYPCP	306760	303370	280160	130	170	210	0.00042	0.00056	0.00075	0.00058	1	2	2	2	0.5
	PCP	121330	145490	172800	130	170	290	0.00107	0.00117	0.00168	0.00131	3	4	5	4	1.0
	Met	173750	179090	158860	180	220	210	0.00104	0.00123	0.00132	0.00120	3	4	4	4	0.5
	MetY	284940	361920	361530	350	440	460	0.00123	0.00122	0.00127	0.00124	4	4	4	4	0.1
	YPCP	278550	268710	260290	370	220	330	0.00133	0.00082	0.00127	0.00114	4	3	4	4	0.9
	Y	192870	293460	313440	230	330	440	0.00119	0.00112	0.00140	0.00124	4	3	4	4	0.5
	X	39720	500870	381910	20	340	230	0.00050	0.00068	0.00060	0.00059	2	2	2	2	0.3
	MV-V	392070	472870	431490	140	230	230	0.00036	0.00049	0.00053	0.00046	1	2	2	1	0.3
IFN	EV	186360	188650	207150	5390	6380	7110	0.02892	0.03382	0.03432	0.03235	89	105	106	100	9.2
	ORF1	331090	407430	411700	11090	13640	15280	0.03350	0.03348	0.03711	0.03470	104	103	115	107	6.5
	MetYPCP	302210	317880	370720	1800	1470	1820	0.00596	0.00462	0.00491	0.00516	18	14	15	16	2.2
	PCP	151110	157330	167870	4460	4000	4730	0.02951	0.02542	0.02818	0.02771	91	79	87	86	6.4
	Met	199150	181240	173830	8380	9060	8410	0.04208	0.04999	0.04838	0.04682	130	155	150	145	12.9
	MetY	404720	417510	428310	7850	7970	7740	0.01940	0.01909	0.01807	0.01885	60	59	56	58	2.1
	YPCP	307040	306240	338200	6900	7350	7990	0.02247	0.02400	0.02363	0.02337	69	74	73	72	2.5
	Y	341030	353540	344960	10160	12200	10530	0.02979	0.03451	0.03053	0.03161	92	107	94	98	7.8
	X	606560	569470	711570	10210	8720	11720	0.01683	0.01531	0.01647	0.01621	52	47	51	50	2.5
	MV-V	540820	599960	571560	510	600	460	0.00094	0.00100	0.00080	0.00092	3	3	2	3	0.3
Experiment 4																
NT	EV	137220	149460	135880	170	220	180	0.00124	0.00147	0.00132	0.00135	2	2	2	2	0.2
	ORF1	189840	186430	187930	2180	4070	2210	0.01148	0.02183	0.01176	0.01502	19	37	20	25	10.0
	MetYPCP	127670	126210	147810	130	260	270	0.00102	0.00205	0.00183	0.00163	2	3	3	3	0.9
	PCP	91050	101560	84190	150	170	130	0.00185	0.00167	0.00154	0.00162	3	3	3	3	0.1
	Met	88370	92430	91270	210	170	190	0.00238	0.00184	0.00208	0.0021	4	3	4	4	0.5
	MetY	117910	97530	121100	320	190	240	0.00271	0.00195	0.00198	0.00221	5	3	3	4	0.7
	YPCP	108680	108150	94910	160	210	290	0.00147	0.00194	0.00306	0.00216	2	3	5	4	1.4
	Y	183030	136090	171300	410	570	530	0.00224	0.00419	0.00309	0.00317	4	7	5	5	1.7
	X	27010	115520	133490	40	150	360	0.00148	0.0013	0.0027	0.00183	3	2	5	3	1.3
	MV-V	134080	124570	127210	180	160	200	0.00134	0.00128	0.00157	0.0014	2	2	3	2	0.3
IFN	EV	170650	169920	149520	9170	9290	10250	0.05374	0.05467	0.06855	0.05899	91	93	116	100	14.1
	ORF1	229380	2467													

Table S1. Raw data from the luciferase reporter assays presented in Figure 1c. Mean ratios between firefly and *Renilla* luciferase activities were calculated (F/R) and are presented as percentages (%) of the treated EV control in 4 independent experiments. Mean values and standard deviation (S.D.) are presented for not-treated (NT) and IFN- β -treated (IFN) conditions.

		Renilla (RLU)			Firefly (RLU)			Firefly/Renilla (F/R)			Mean (F/R)	%			Mean (%)	S.D. (%)
Experiment 1																
NT	EV	200300	201930	193190	300	340	450	0.0015	0.0017	0.0023	0.0018	2	3	4	3	0.7
	MetYPCP-G3	264980	282670	235400	290	320	190	0.0011	0.0011	0.0008	0.0010	2	2	1	2	0.3
	PCP-G3	177850	200810	186360	220	220	350	0.0012	0.0011	0.0019	0.0014	2	2	3	2	0.7
	MetYPCP-G1	202530	207220	222490	360	310	300	0.0018	0.0015	0.0013	0.0015	3	2	2	2	0.3
	PCP-G1	148850	212410	210900	260	260	360	0.0017	0.0012	0.0017	0.0016	3	2	3	2	0.5
	MV-V	271880	268710	256650	200	300	220	0.0007	0.0011	0.0009	0.0009	1	2	1	1	0.3
IFN	EV	216550	222760	207050	14800	13890	11800	0.0683	0.0624	0.0570	0.0626	109	100	91	100	9.1
	MetYPCP-G3	328990	291910	280530	6050	5840	6230	0.0184	0.0200	0.0222	0.0202	29	32	35	32	3.1
	PCP-G3	214980	231230	233310	8470	9380	8770	0.0394	0.0406	0.0376	0.0392	63	65	60	63	2.4
	MetYPCP-G1	268250	245390	287010	10810	8390	10550	0.0403	0.0342	0.0368	0.0371	64	55	59	59	4.9
	PCP-G1	245420	228780	221960	16700	18660	14520	0.0680	0.0816	0.0854	0.0717	109	130	105	115	13.8
	MV-V	314250	303040	312720	740	930	760	0.0024	0.0031	0.0024	0.0026	4	5	4	4	0.6
Experiment 2																
NT	EV	70170	66930	65930	130	80	150	0.00185	0.0012	0.00228	0.0018	9	6	11	9	2.7
	MetYPCP-G3	36800	29700	44740	60	60	60	0.00163	0.00202	0.00134	0.0017	8	10	7	8	1.7
	PCP-G3	48340	43100	50030	40	90	20	0.00083	0.00209	0.0004	0.0011	4	10	2	5	4.3
	MetYPCP-G1	45740	37020	43580	60	90	100	0.00131	0.00243	0.00229	0.0020	6	12	11	10	3.0
	PCP-G1	60430	54670	61390	80	70	70	0.00132	0.00128	0.00114	0.0012	7	6	6	6	0.5
	MV-V	35540	40000	30880	90	100	20	0.00253	0.0025	0.00065	0.0019	13	12	3	9	5.3
IFN	EV	81100	90050	87250	1540	1610	2080	0.01899	0.01788	0.02384	0.02024	94	88	118	100	15.7
	MetYPCP-G3	51430	60800	52650	790	800	740	0.01536	0.01316	0.01406	0.01419	76	65	69	70	5.5
	PCP-G3	46860	43900	46970	970	1190	1270	0.0207	0.02711	0.02704	0.02495	102	134	134	123	18.2
	MetYPCP-G1	54080	60920	56870	1440	1730	1420	0.02663	0.0284	0.02497	0.02666	132	140	123	132	8.5
	PCP-G1	59910	66630	63450	1380	1420	1780	0.02303	0.02131	0.02805	0.02413	114	105	139	119	17.3
	MV-V	49480	75870	57270	100	130	60	0.00202	0.00171	0.00105	0.00159	10	8	5	8	2.5
Experiment 3																
NT	EV	202890	217220	173590	250	450	250	0.00123	0.00207	0.00144	0.00158	4	6	4	5	1.4
	MetYPCP-G3	306760	303370	280160	130	170	210	0.00042	0.00056	0.00075	0.00058	1	2	2	2	0.5
	PCP-G3	121330	145490	172800	130	170	290	0.00107	0.00117	0.00168	0.00131	3	4	5	4	1.0
	MetYPCP-G1	247460	244000	277580	160	130	180	0.00065	0.00053	0.00065	0.00061	2	2	2	2	0.2
	PCP-G1	355340	499600	352410	260	360	240	0.00073	0.00072	0.00068	0.00071	2	2	2	2	0.1
	MV-V	392070	472870	431490	140	230	230	0.00036	0.00049	0.00053	0.00046	1	2	2	1	0.3
IFN	EV	186360	186650	207150	5390	6380	7110	0.02892	0.03382	0.03432	0.03235	89	105	106	100	9.2
	MetYPCP-G3	302210	317880	370720	1800	1470	1820	0.00596	0.00462	0.00491	0.00516	18	14	15	16	2.2
	PCP-G3	151110	157330	167870	4460	4000	4730	0.02951	0.02542	0.02818	0.02771	91	79	87	86	6.4
	MetYPCP-G1	335600	282950	299520	3110	3240	3230	0.00927	0.01145	0.01078	0.01050	29	35	33	32	3.5
	PCP-G1	412860	393580	372060	6850	5680	5980	0.01659	0.01443	0.01607	0.01570	51	45	50	49	3.5
	MV-V	540820	599960	571560	510	600	460	0.00094	0.00100	0.00080	0.00092	3	3	2	3	0.3
Experiment 4																
NT	EV	137220	149460	135880	170	220	180	0.00124	0.00147	0.00132	0.0013452	2	2	2	2	0.2
	MetYPCP-G3	127670	126210	147810	130	260	270	0.00102	0.00206	0.00183	0.001635	2	3	3	3	0.9
	PCP-G3	91050	101560	84190	150	170	130	0.00165	0.00167	0.00154	0.0016218	3	3	3	3	0.3
	MetYPCP-G1	298560	298620	303850	310	240	210	0.00104	0.0008	0.00069	0.0008444	2	1	1	1	0.3
	PCP-G1	121710	112650	111290	300	310	440	0.00246	0.00275	0.00395	0.0030568	4	5	7	5	1.3
	MV-V	134080	124570	127210	180	160	200	0.00134	0.00128	0.00157	0.0013997	2	2	3	2	0.3
IFN	EV	170650	169920	149520	9170	9290	10250	0.05374	0.05467	0.06855	0.0589871	91	93	116	100	14.1
	MetYPCP-G3	137030	138520	121100	5190	5020	5660	0.03787	0.03624	0.04674	0.0402845	64	61	79	68	9.6
	PCP-G3	111450	105190	112120	4750	3720	4160	0.04262	0.03536	0.0371	0.0383626	72	60	63	65	6.4
	MetYPCP-G1	312570	304910	274430	10090	8540	9880	0.03228	0.02801	0.036	0.032097	55	47	61	54	6.8
	PCP-G1	125860	122480	113670	11860	12790	9390	0.09423	0.10443	0.08261	0.0937548	160	177	140	159	18.5
	MV-V	132770	136310	128020	460	640	520	0.00346	0.0047	0.00406	0.0040739	6	8	7	7	1.0
Experiment 5																
NT	EV	34730	61960	53620	60	250	140	0.00173	0.00403	0.00261	0.0027911	2	5	3	3	1.4
	MetYPCP-G3	106760	106090	114750	180	200	240	0.00169	0.00189	0.00209	0.0018876	2	2	3	2	0.3
	PCP-G3	59960	57460	69510	140	100	160	0.00233	0.00174	0.0023	0.0021257	3	2	3	3	0.4
	MetYPCP-G1	55420	54140	62980	200	250	280	0.00361	0.00462	0.00445	0.0042241	4	6	5	5	0.7
	PCP-G1	46030	54840	50000	190	250	150	0.00413	0.00456	0.003	0.0038955	5	6	4	5	1.0
	MV-V	63230	55010	51370	120	100	100	0.0019	0.00182	0.00195	0.0018874	2	2	2	2	0.1
IFN	EV	70050	69220	61310	5650	5120	5390	0.08066	0.07397	0.08791	0.0808459	100	91	109	100	8.6
	MetYPCP-G3	130380	125220	99210	4130	3710	3680	0.03168	0.02963	0.03709	0.0327992	39	37	46	41	4.8
	PCP-G3	84470	70890	55510	4420	4310	3980	0.05233	0.0608	0.0717	0.0616078	65	75	89	76	12.0
	MetYPCP-G1	68850	72360	60140	5070	5070	4430	0.07364	0.07007	0.07366	0.0724554	91	87	91	90	2.6
	PCP-G1	66090	56780	53050	5350	3860	4170	0.08095	0.06798	0.07861	0.0758457	100	84	97	94	8.5
	MV-V	58160	49940	53740	750	550	670	0.0129	0.01101	0.01247	0.0121254	16	14	15	15	1.2

Table S2. Raw data from the luciferase reporter assays presented in Figure 6b. Mean ratios between firefly and *Renilla* luciferase activities were calculated (F/R) and are presented as percentages (%) of the treated EV control in 5 independent experiments. Mean values and standard deviation (S.D.) are presented for not-treated (NT) and IFN- β -treated (IFN) conditions.

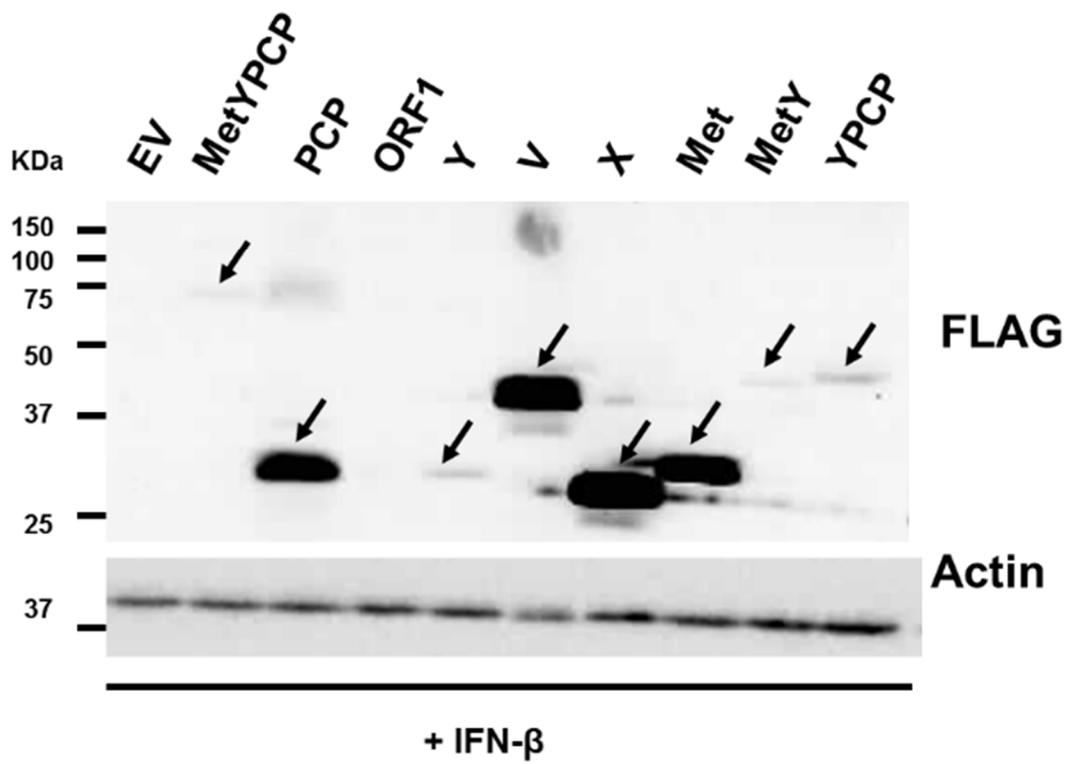
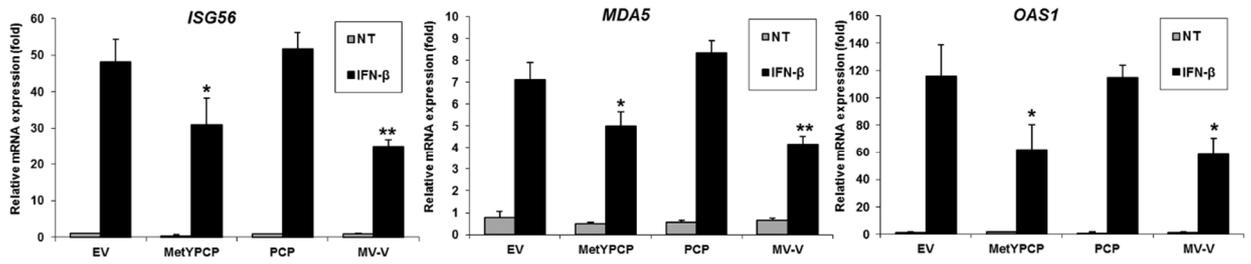
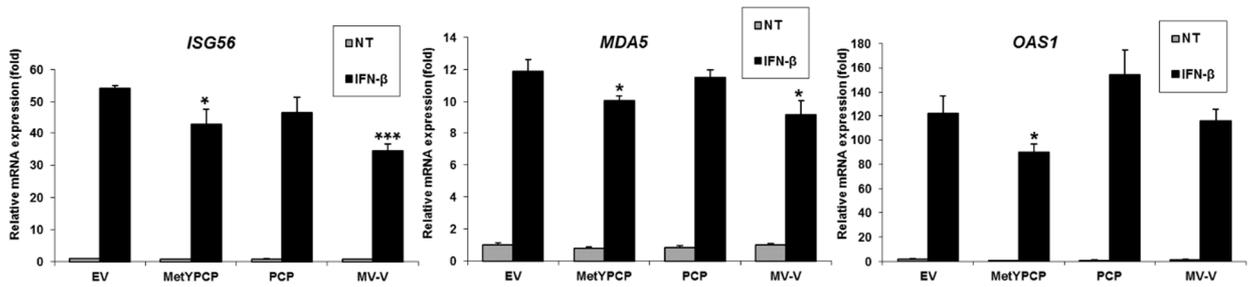


Figure S1. Expression of FLAG-tagged full-length and domains of ORF1 in co-transfected 293T cells. Cell lysates generated for the luciferase reporter assay (Figure 1c) were analysed by immunoblotting using an anti-FLAG antibody. Bands corresponding to the different FLAG-tagged proteins are shown by arrows. Note that ORF1 was not detected. Actin served as loading control.

Experiment 1



Experiment 2



Experiment 3

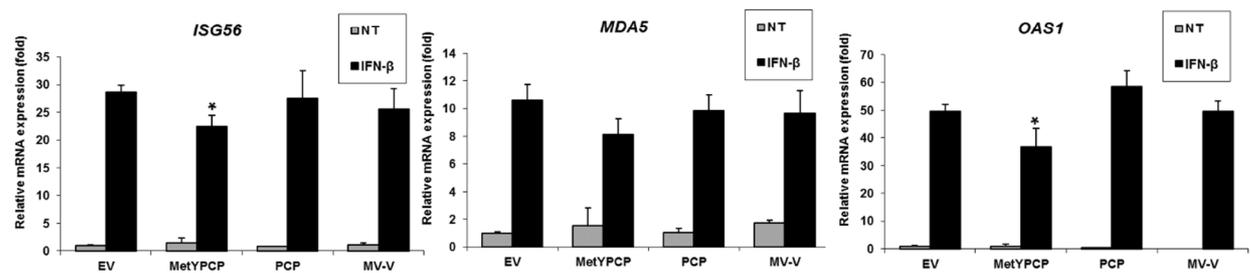


Figure S2. Relative expression of ISG56, MDA5 and OAS1 mRNA determined by RT-qPCR for each of the 3 independent experiments presented in Figure 2. Data are presented as fold induction (+ standard deviations) relative to the non-stimulated EV control. *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.0005$ compared to EV control for treated samples (unpaired t tests).

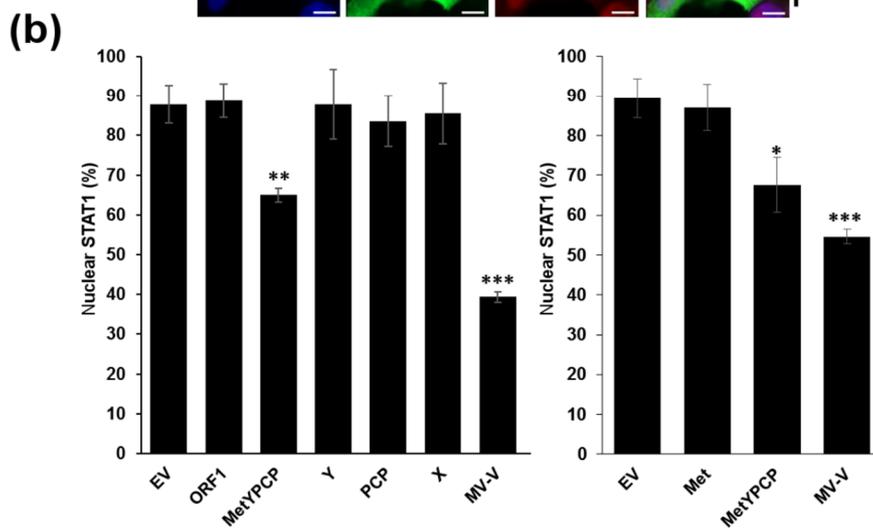
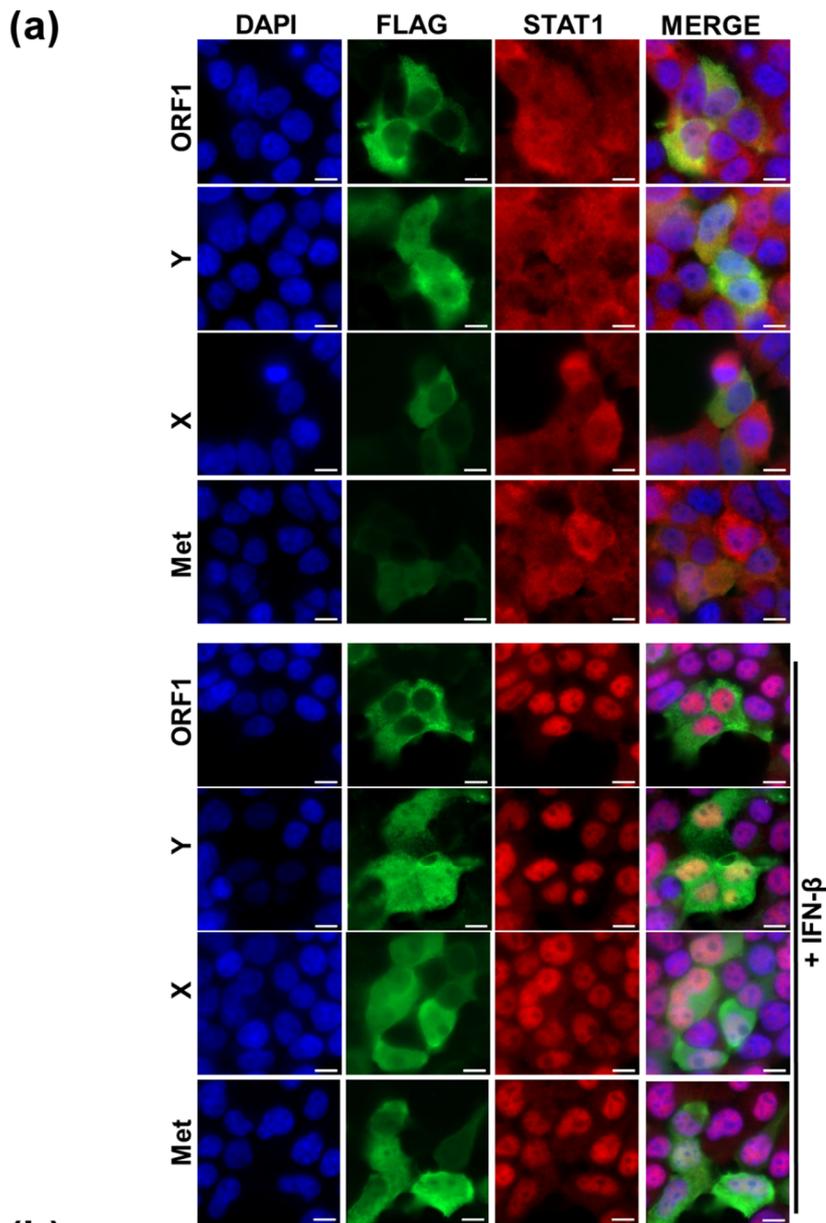


Figure S3. Expression of ORF1, Y, X and Met of HEV ORF1 has no impact on STAT1 nuclear translocation upon IFN- β treatment. (a) 293T cells were transfected with a pCINeo-3xFLAG empty vector (EV) or a plasmid coding for ORF1, Y, X or Met fused to a FLAG tag. Twenty four h post-transfection, cells were left untreated or stimulated for 30 min with 1000 IU/ml of IFN- β . Cells were then washed, fixed and stained with primary antibodies raised against STAT1 and FLAG, followed by fluorescent dye-conjugated secondary antibodies. Intracellular localization of DAPI-stained nuclei (blue), FLAG (green) and STAT1 (red) was visualized by microscopy (magnification, x630). Scale bars, 10 μ m. (b) STAT1 localization was visualized after immunostaining as described in (a) in 293T cells transfected with a pCINeo-3xFLAG empty vector (EV) or a plasmid coding for ORF1, MetYPCP, PCP, X, Y, Met or MV-V fused to a FLAG tag. For each condition, STAT1 localization was determined in 48 to 181 cells expressing the corresponding FLAG-tagged protein (except for the EV control for which 356 to 384 cells were randomly assessed). The mean percentage (\pm standard deviation) of cells showing a predominant nuclear localization of STAT1 from 3 independent experiments is shown. * p <0.05; ** p <0.005; *** p <0.0005 compared to EV control for treated samples (unpaired t tests). (a-b) Data presented for the Met domain are from a different set of experiments than the one presented for ORF1, Y and X.

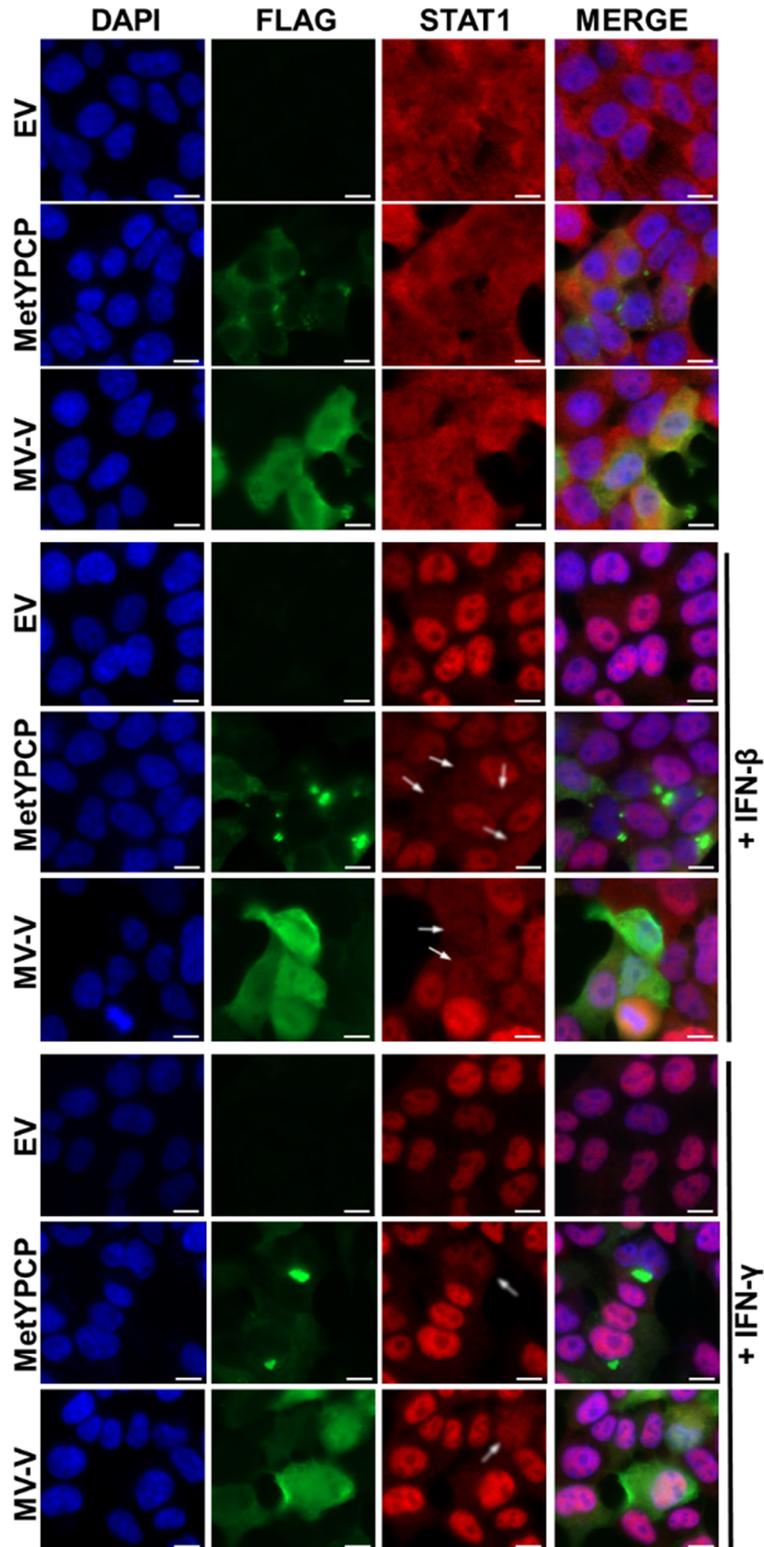


Figure S4. Effect of MetYPCP of HEV ORF1 on STAT1 translocation in response to IFN-II. 293T cells were transfected with a pCINeo-3×FLAG empty vector (EV) or a plasmid coding for MetYPCP or MV-V fused to a 3×FLAG tag. Twenty four h post-transfection, cells were stimulated for 30 min with 1000 IU/ml of IFN- β or 250 ng/ml of IFN- γ . Cells were then washed, fixed and stained with primary antibodies raised against STAT1 and FLAG, followed by fluorescent dye-conjugated secondary antibodies. Cells showing diffuse cytoplasmic/nuclear localisation of STAT1 upon IFN- β treatment are indicated by arrows. Scale bars, 10 μ m.

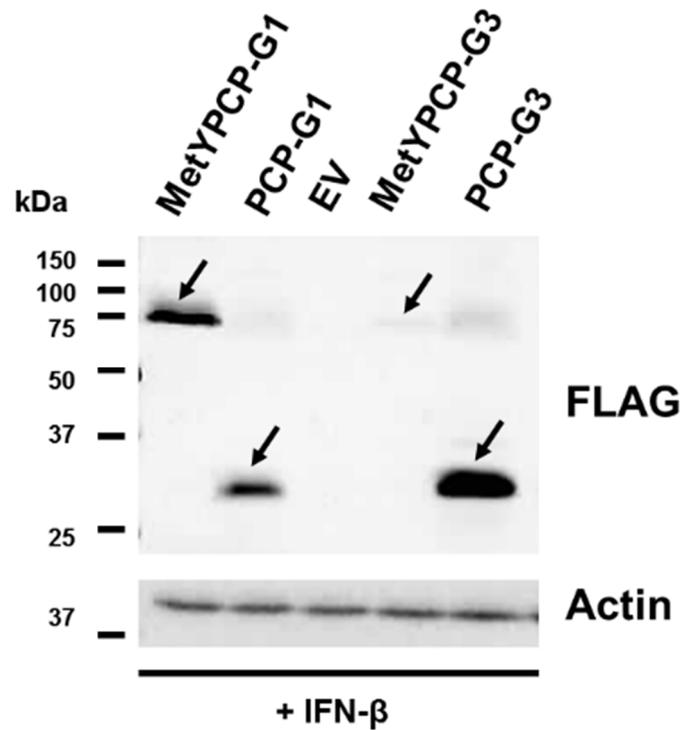


Figure S6. Expression of FLAG-tagged MetYPCP and PCP from genotypes 1 and 3 in co-transfected 293T cells. Cell lysates generated for the luciferase reporter assay (Figure 6b) were analysed by immunoblotting using an anti-FLAG antibody. Bands corresponding to the different FLAG-tagged proteins are shown by arrows. Actin served as loading control.

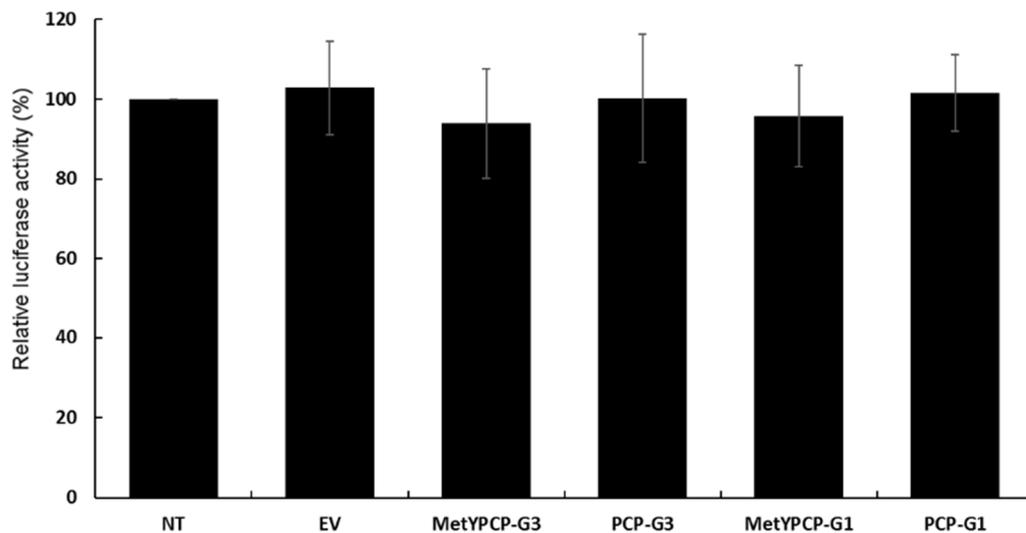


Figure S7. Cell viability assay in cells expressing MetYPCP and PCP from HEV-1 and HEV-3. 293T cells were transfected or not (NT) with a pCINeo-3xFLAG empty vector (EV) or a plasmid coding for MetYPCP and PCP from genotype 1 and 3 fused to a 3xFLAG tag. Forty h after transfection, cells were lysed and cell viability determined using a luminescent-based assay. Luciferase activities (\pm standard deviations) are expressed as percentage relative to non-transfected cells. No significant difference was found between the cells transfected with the pCINeo-3xFLAG empty vector and the one transfected with the plasmids coding for MetYPCP or PCP from HEV-1 and HEV-3 (unpaired t tests). Results represent the mean of 3 independent experiments performed in triplicate.

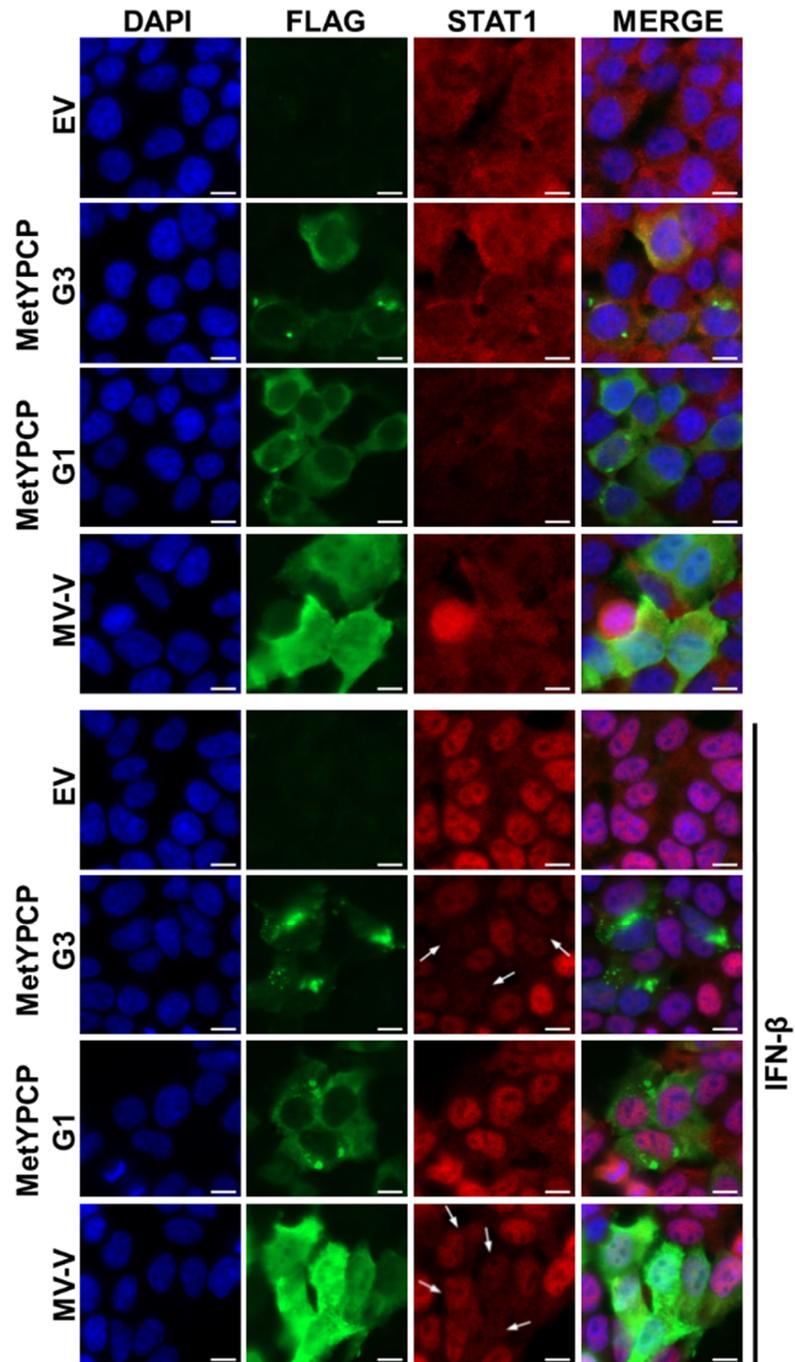


Figure S8. Effect of MetYPCP from HEV-1 on STAT1 translocation. 293T cells were transfected with a pCIneo-3xFLAG empty vector (EV) or a plasmid coding for MetYPCP from HEV-1 or HEV-3 or MV-V fused to a 3xFLAG tag. Twenty-four h post-transfection, cells were stimulated for 30 min with 1000 IU/ml of IFN- β . Cells were then washed, fixed and stained with primary antibodies raised against STAT1 and FLAG, followed by fluorescent dye-conjugated secondary antibodies. Cells showing diffuse STAT1 cytoplasmic/nuclear localisation upon IFN- β treatment are indicated by arrows. Scale bars, 10 μ m.