

The IFITM proteins inhibit HIV-1 infection

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Supplementary Table S1. Primer sequences

IFITM1-S	5'-GCACGGATCCATGGATTACAAGGATGACGACGATAAAGCACAAGGAGGAACATGAGG-3'
IFITM1-A	5'-GCACGAATTCCTAGTAACCCCGTTTTTCC-3'
IFITM2-S	5'-GCACGGATCCATGGATTACAAGGATGACGACGATAAGAACCACATTGTGCAAACCTT-3'
IFITM2-A	5'-GCACGAATTCCTATCGCTGGGCTGGAC-3'
IFITM3-S	5'-GCACGGATCCATGGATTACAAGGATGACGACGATAAGAATCACACTGTCCAAACCTT-3'
IFITM3-A	5'-GCACGAATTCCTATCCATAGGCTGGAAG-3'
TM1(117-125)-A	5'-GCACGAATTCCTATAACATAATATGGTAGACTGTCC-3'
TM1(112-125)-A	5'-GCACGAATTCCTAGACTGTCCAGAGCCGAATAC-3'
TM1(108-125)-A	5'-GCACGAATTCCTAGCCGAATACCAGTAACAGGAT-3'
(1-21)-S	5'-GCACGGATCCATGGATTACAAGGATGACGACGATAAGTCCACCGTGATCAACATCCAC-3'
(1-29)-S	5'-GCACGGATCCATGGATTACAAGGATGACGACGATAAGGAGACCTCCGTGCCCCGACCAT-3'
SVKS	5'-GCATTCGCCTACGCCGCGGCGGCTAGGGACAGGAAG-3'
RDRK	5'-CTCCGTGAAGTCTGCGGCCGCGGCGATGGTTGGCGAC-3'
MVGD	5'-CTAGGGACAGGAAGGCGGCTGCCGCCGTGACCGGGGC-3'
VTG	5'-GATGGTTGGCGACGCGGCCGCGGCCAGGCC-3'
QY	5'-GACCGGGGCCGCGGCCGCTGCCTCCACCG-3'
ST	5'-CAGGCCTATGCCGCCGCCGCAAGTGCCTG-3'
KC	5'-GCCTCCACCGCCGCGGCCCTGAACATCTGG-3'
LNI	5'-ACCGCCAAGTGC GCGGCCGCTGGGCCCTGATTC-3'
TM3(1-6)	5'-GCACGGATCCATGGATTACAAGGATGACGACGATAAGACCTTCTTCTCTCTCTGTCAAC -3'
TM3(1-11)	5'-GCACGGATCCATGGATTACAAGGATGACGACGATAAGGTCAACAGTGGCCAGCCCCCAAC-3'
TM3(1-16)	5'-GCACGGATCCATGGATTACAAGGATGACGACGATAAGCCCCCAACTATGAGATGCTC-3'

Supplementary Table S2. ISGs up-regulated in SupT1 cells. ISGs that were tested in this study are marked on a separate column.

Target ID	Folds change 8h	Folds change 16h	Tested in this study
MX1	115.639142	141.8609791	x
IFI44L	71.73632829	67.38894337	
EPSTI1	60.75536894	37.99656248	x
IFIT1	51.22339605	26.80311923	x
IFIT2	49.90273563	14.30525193	x
IFIT3	46.04341705	26.52318271	x
IFI44	39.38842978	43.20951103	x
PRIC285	34.6421124	9.482687546	x
MT2A	29.08227046	10.06330298	x
IFI6	29.03795601	25.57222876	x
HERC5	20.29071784	8.657845165	x
MT1A	18.25941956	6.5735696	
IFI35	18.14708602	9.219260288	x
STAT1	16.00772075	11.06140699	x
IFIH1	15.90905239	7.396967823	
IFITM1	13.5434276	7.133242402	x
RSAD2	13.01883095	5.66283306	x
ISG15	12.38882965	10.50106685	x
HERC6	12.28294838	8.991962382	x
OAS2	12.23733922	12.32141465	x
GBP1	12.15015657	3.421236887	x
IRF7	12.07405188	6.061572047	x
TAP1	11.67759276	4.050693707	x
IFI27	11.33412411	14.17574842	x
GBP2	11.1589084	3.040414458	x
SAMD9	10.45797774	5.188922542	x
BST2	10.37792421	9.979862925	x
OAS1	10.27809509	6.045061429	x
PLSCR1	10.23856059	7.003693747	x
HS.125087	10.05601638	10.43703487	
SP110	9.842480847	3.948717068	x
FLJ20035	9.76066877	10.24176176	
ISG20	9.366123102	5.244030222	x
PARP9	9.170967879	9.68033517	x
EIF2AK2	9.022304641	6.836396152	
SAMD9L	8.907644611	4.137218061	
PARP12	8.063515743	4.115923021	x
G1P3	8.036837991	10.37103519	
OASL	7.931473978	2.731485946	x
MAFA	7.921282337	7.424974134	
MX2	7.023070902	3.219103583	x
TRIM22	6.979240273	3.339416405	x
DDX58	6.947384765	2.527713583	x
RASGRP3	6.911040818	2.325993395	
ISGF3G	6.852084771	3.720202105	
XAF1	6.391147573	4.398001207	x
CDA	6.02833253	5.185974096	x
PARP14	6.01409226	3.427762137	x
HLA-B	5.613019222	3.97970088	
NT5C3	5.606156427	2.977746623	x

STAT2	5.547189458	3.038202436	x
RARRES3	5.488755215	4.052479815	x
LGP2	5.43188524	3.158273257	x
LBA1	5.279132952	2.419681232	
GZMB	5.117524131	2.113508625	x
DTX3L	4.997047736	2.977274001	x
PSMB9	4.938979566	2.618858298	x
DNAPTP6	4.894718868	3.032975828	x
USP18	4.805738008	2.646197974	x
RTP4	4.550130742	2.987983124	x
NMI	4.505183908	3.072708695	x
LY6E	4.457784886	2.874238939	x
TDRD7	4.425556865	2.427913461	x
LAP3	4.424814577	2.410265377	x
KIAA1618	4.373162628	3.295930908	x
PARP10	4.338898989	2.569858634	x
SP100	4.193820169	2.461624999	x
LYSMD2	4.088016445	2.50455449	
FLJ11286	4.053314164	2.636708347	
IFIT5	3.966350028	3.073040968	x
LOC129607	3.872904696	2.206989858	
ZNFX1	3.770842778	2.136700944	
TRIM25	3.7487432	2.187745622	
LGALS3BP	3.63971377	2.114305721	
TAP2	3.630343346	2.007628038	
PSMB8	3.200787723	2.420835418	
UBE2L6	3.186916015	2.03353948	x
MYD88	3.142115036	2.131624027	x
HS.489254	3.114360604	2.484080626	
ADAR	3.041398799	2.31871901	x
PSME2	2.97171343	3.085041721	
HDHD1A	2.427231982	2.014176346	
NME1	2.316054175	2.533126281	
CD38	2.311020408	2.167155988	
TFRC	2.289484821	3.212514576	
SLA	2.231633324	2.413212566	
NOLC1	2.218499149	2.246988904	
HLA-H	2.18634489	2.308470026	
PTPN6	2.17877014	2.641651223	
FAM98A	2.124524528	2.180614884	
B2M	2.083475305	2.243879184	
PSMD1	2.069739213	2.059796769	
PSMA3	2.061183573	2.251154384	
RGPD1	2.031485405	2.872422563	

Supplementary Figure S1. Effects of IFN α 2b on HIV-1 production in SupT1 (A) and CBMCs (B). Cells were treated with IFN α 2b for 16 hours before infection with wild type HIV-1. Viral Gag expression in cells was assessed by Western blotting using anti-HIV-1 p24 antibody. Levels of HIV-1 in the supernatants were determined by measuring viral reverse transcriptase activity or by infecting the TZM-bl indicator cells. Results shown are representative of two independent experiments. HIV-1 production from Jurkat, U937 and 293T cells was affected by IFN α 2b to a much less pronounced extent (data not shown).

Supplementary Figure S2. Characterization of doxycycline-inducible SupT1 cell lines. (A) Cell proliferation. Each SupT1 cell line was seeded in 24-well plates in triplicate with or without doxycycline (500 ng/ml). Cell numbers were scored at different time intervals. (B) Cell surface expression of CD4. Cells were stained with PE-conjugated anti-human CD4 antibodies on ice for 30 minutes before being fixed and analyzed by flow cytometry.

Supplementary Figure S3. IFITM proteins protect MT-2 cells from HIV-1 infection. (A) Doxycycline-induced expression of IFITM proteins in MT-2 cell lines as being measured by Western blotting. (C) Infection of MT-2 cell lines with NLEY1-IRES virus. Number of YFP-positive cells was scored by flow cytometry. Amounts of infectious HIV-1 particles in supernatants were determined by infecting the TZM-bl indicator cells. Folds of change were calculated by dividing the values in the presence of doxycycline (+Dox) with the values in the absence of doxycycline (-Dox) for each shRNA cell line.

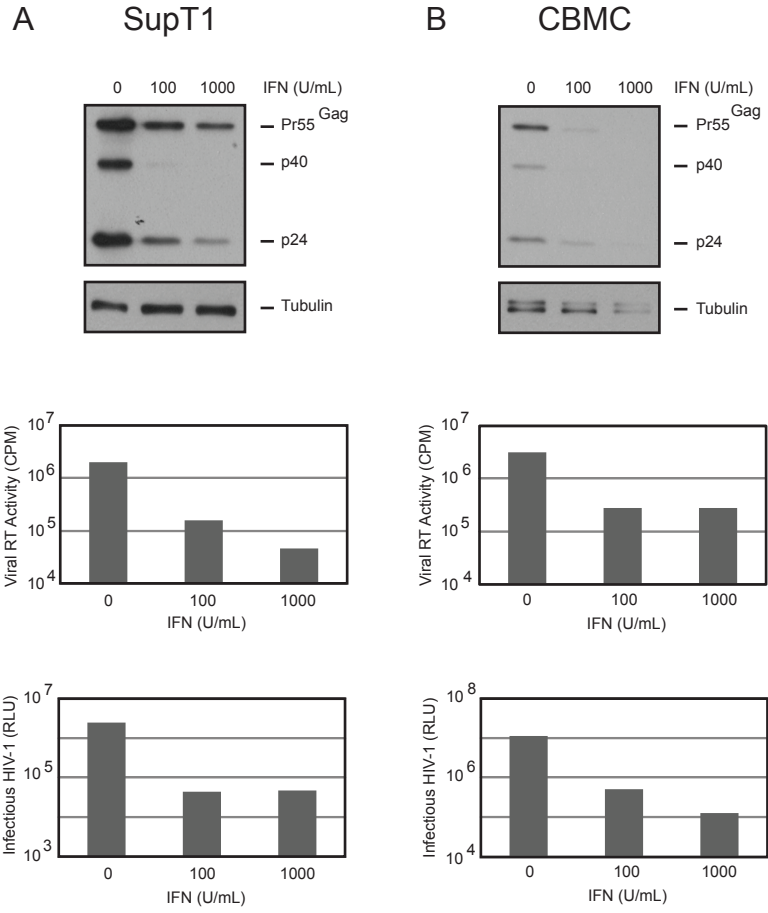


Figure S1

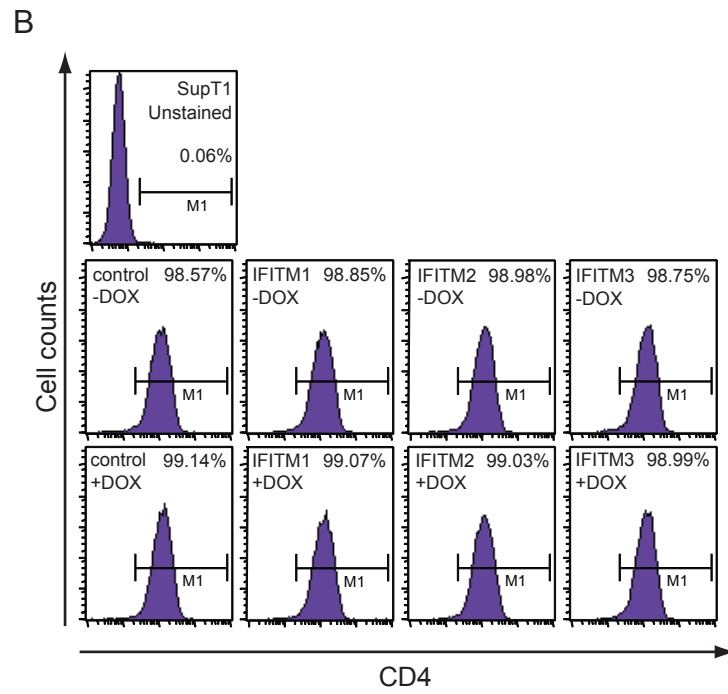
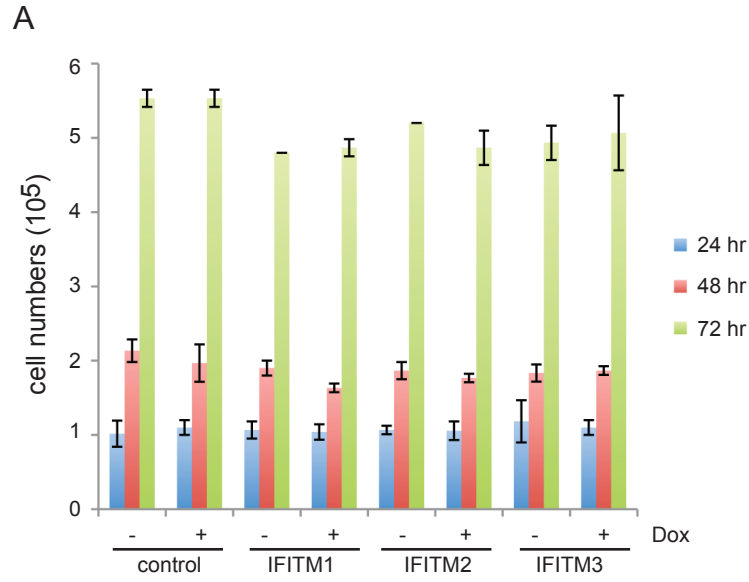


Figure S2

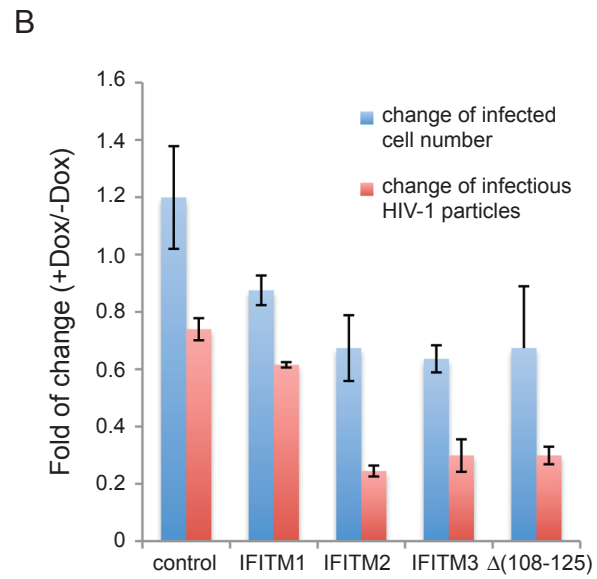
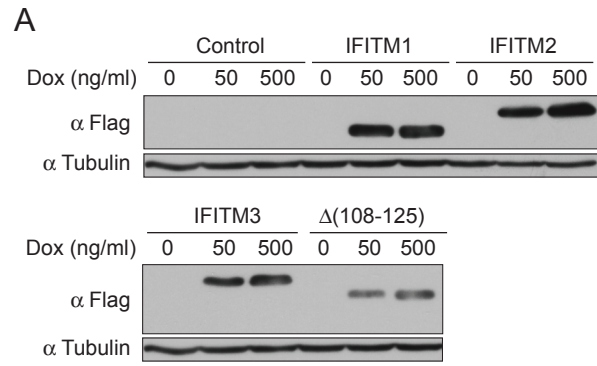


Figure S3