Supporting Online Information

REGULATION OF IRF-3-DEPENDENT INNATE IMMUNITY BY THE PAPAIN-LIKE PROTEASE DOMAIN OF THE SARS CORONAVIRUS

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1. Humanized cDNA sequence of SARS CoV PLpro core domain.

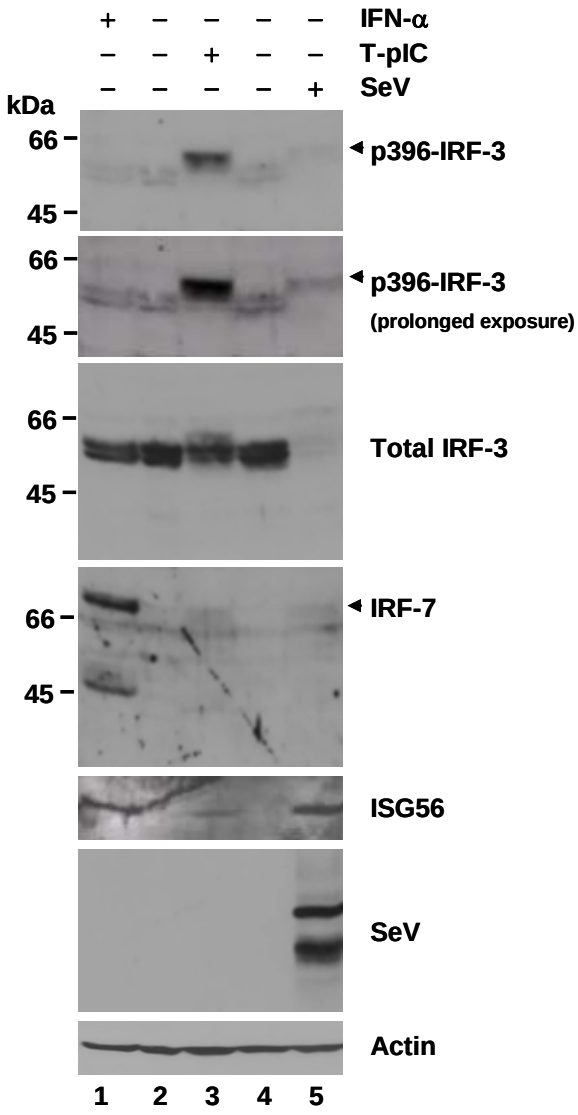
Bam HI and EcoRI restriction sites were added to both 5' and 3' ends of the cDNA for cloning into pcDNA 3.1-V5/His B. Kozak sequence and start codon were added to 5' end for efficient translation in eukaryotic cells.

ggatccactATGGAGGTGAAGACCATCAAGGTGTTCACCACCGTGGACAACACCAACCTG
CACACCCAGCTGGTGGACATGAGCATGACCTACGGCCAGCAGTTCGGCCCCACCTACCTG
GACGGCGCCGACGTGACCAAGATCAAGCCCCACGTGAACCACGAGGGCAAGACCTTCTTC
GTGCTGCCCAGCGACGACACCCTGCGGAGCGAGGCCTTCGAGTACTACCACACCCTGGAC
GAGAGCTTCCTGGGCAGATACATGAGCGCCCTGAACCACACCAAGAAGTGGAAGTTCCCC
CAAGTCGGCGGCCTGACCAGCATCAAGTGGGCCGACAACAACTGCTACCTGAGCAGCGTG
CTGCTGGCCCTGCAGCAGCTGGAAGTCAAGTTCAACGCCCCCGCCCTGCAAGAGGCCTAC
TACCGGGCCAGGGCCGGCGACGCCGCCAACTTCTGCGCCCTGATCCTGGCCTACTCCAAC
AAGACCGTGGGCGAGCTGGGCGACGTGCGGGAGACCATGACCCACCTGCTGCAGCACGCC
AACCTGGAGAGCGCCAAGCGGGTGCTGAACGTGGTGTGCAAGCACTGCGGCCAGAAGACC
ACCACCCTGACCGGCGTGGAGGCCGTGATGTACATGGGCACCCTGAGCTACGACAACCTG
AAGACCGGGGTCAGCATCCCCTGCGTGTGCGGCCGGGACGCCACCCAGTACCTGGTGCAG
CAGGAGAGCAGCTTCGTGATGATGAGCGCCCCCCCCGCCGAGTACAAGCTGCAGCAGGGC
ACCTTCCTGTGCGCCAACGAGTACACCGGCAACTACCAGTGCGGCCACTACACCCACATC
ACCGCCAAGGAGACCCTGTACCGGATCGACGGCGCCCACCTGACCAAGATGAGCGAGTAC
AAGGGCCCCGTGACCGACGTGTTCTACAAGGAGACCAGCTACACCACCACCATCAAGTG**g**
aattc

2. Primers for site-directed mutagenesis of SARS-CoV PLpro.

Mutant	Primers	Oligonucleotide sequence (5' to 3')
PLpro C1651A	P1	5' GCC GAC AAC AAC GCC TAC CTG AGC AGC 3'
	P2	5' GCT GCT CAG GTA GGC GTT GTT GTC GGC 3'
PLpro D1826A	P1	5' CGG ATC GCT GGC GCC CAC CTG ACC AAG ATG AGC GAG 3'
	P2	5' GGC GCC AGC GAT CCG GTA CAG GGT CTC CTT GGC GG 3'

Devaraj et al, Suppl. Fig.1



Supplemental Fig.1. MA104 cells grown in 35-mm dishes were treated with 1000 IU/ml of recombinant human IFN-α2b for 24 h (lane 1), mock-treated (lane 2 and 4) , transfected with 5 μg poly (I-C) (T-pIC, lane 3) for 8 h, or infected with 100 HAU/ml of SeV for 8 h (lane 5), prior to cell lysis and immunoblot analysis of p396-IRF3, IRF-3, IRF-7, ISG56, SeV, or actin. Note that the p396-IRF3 abundance in SeV-infected cells is much lower that that induced by T-pIC, due to SeV-induced degradation of IRF-3. IRF-7 is not basally expressed in MA104 cells, and its expression is minimally induced by SeV or T-pIC during this short period of treatment (8 h). The MW of IRF-7 is higher than that of p396-IRF3.

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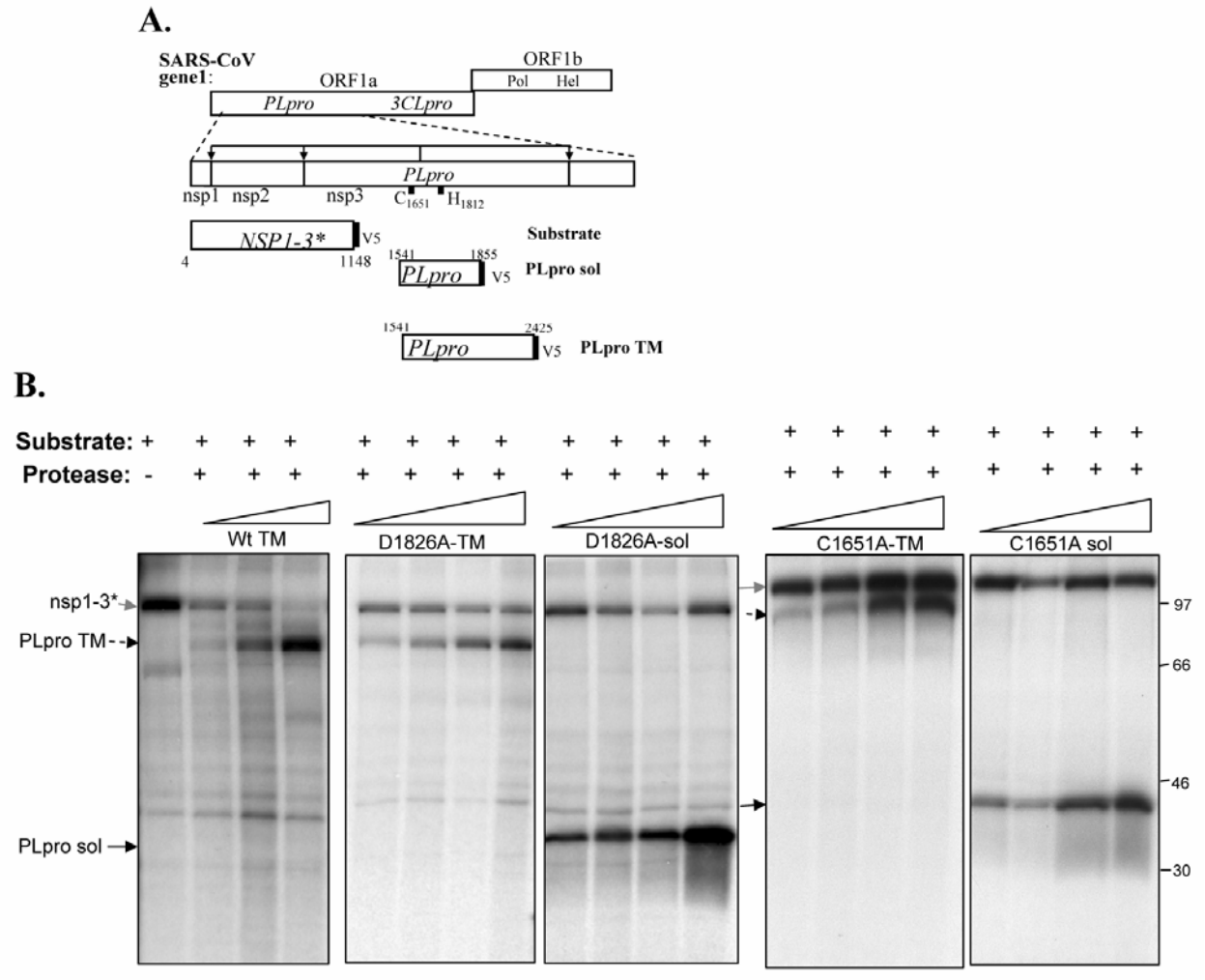

<u>Supplemental Fig.2</u>. Protease activity of various SARS-CoV PLpro mutants.

A. Schematic diagram of constructs expressing PLpro and substrate NSP1-3*. The catalytic cysteine, histidine and aspartic acid residues are indicated as are the residues at which they begin and end. Figure adapted from Barretto et al (JVI, 2005).

B. Trans-cleavage assay to assess proteolytic activity of PLpro Wt, D1826A and C1651A mutants. Increasing amounts of soluble (sol) or transmembrane (TM) versions of SARS-CoV PLpro Wt, D1826A or C1651A mutant, were co-expressed along with substrate nsp1-3*, via vaccinia-driven T7 expression in HeLa cells. The proteins were radiolabeled with Trans-^{35}S, immunoprecipitated with anti-V5, which precipitates the uncleaved substrate nsp1-3* as well as the protease. Products were analyzed by electrophoresis on 10% SDS-polyacrylamide gels and visualized by autoradiography. Positions of substrate nsp1-3* is indicated by a grey arrow, PLpro TM by a dotted arrow and PLpro sol by a black arrow. Note the disappearance of the nsp1-3 full length substrate for WT but not the D1826A or C1651A mutants.