Supplementary Figure Legends

Figure S1 Identification of PASD1 as a component of the STAT3 activation pathways.

(**A**) Specificity validation of STAT3 reporter. The STAT3 reporter (20 ng) was transfected into HEK293 cells (5×10^4). Twenty hours after transfection, cells were treated with IL-6 (20 ng/mL), LIF (5 ng/mL), INF-β (100 ng/mL), IFN-γ (100 ng/mL) or left untreated in DMEM without FBS for 12 h before reporter assays.

(**B**) Overexpression of PASD1 activates STAT3 and potentiates IL-6-induced STAT3 activation. Human and murine cDNA clones (Origene Inc.) (50 ng) were individually transfected into HEK293 cells (5×10^4) together with STAT3 reporter (20 ng). Twenty hours after transfection, cells were treated with IL-6 (20 ng/mL) or left untreated in DMEM without FBS for 12 h before reporter assays. The histograph shows a part of the screening data.

(**C**) Effects of PASD1 isoforms on STAT3 activation. HEK293 cells (5×10^4) were transfected with STAT3 reporter (20 ng) and expression plasmids for the indicated PASD1 isoforms. Twenty hours after transfection, cells were treated with IL-6 (20 ng/mL) or left untreated for 10 h in DMEM without FBS before luciferase assays were performed. Results are represented as mean ± SD, n=3, $^{**}p < 0.01$.

(**D**) Overexpression of PASD1 potentiates IL-6-induced transcription of endogenous *SOCS3* gene in HEK293 cells. HEK293 (2×10^5) were transfected with an empty vector or PASD1 expression plasmid (0.2 μg). Twenty hours after transfection, cells were starved with DMEM without FBS overnight followed by IL-6 treatment (20 ng/mL) for the indicated times before qPCR experiments were performed.

Figure S2 Expression of PASD1.

(**A**) Expression of endogenous and overexpressed PASD1.

Left panels: The GFP-RNAi control or PASD1-RNAi retrovirus-transduced HeLa cells (1×10^6) were lysed and analyzed by immnuoblot with rabbit antiserum against PASD1. The asterisk denotes non-specific bands.

Right panels: The control vector or Flag-tagged long isoform of PASD1 (0.5 μg) were transfected into HEK29T cells (2×10^5) before immunoblot analysis was performed with the indicated antibodies.

(**B**) Expression profiles of PASD1 in several cell lines. The indicated cell lines were lysed and immunoprecipitation experiments were performed using rabbit antibody against PASD1. Immunoblot analyses were performed with mouse antibody against PASD1.

(**C**) Knockdown efficiency of PASD1-RNAi plasmids. The Flag-tagged PASD1 and STRBP (0.5 μg) were transfected with GFP-RNAi control plasmid and four PASD1-RNAi plasmids into HEK293T cells (2×10^5). Twenty-four hours after transfection, cells were lysed and immunoblot assays were performed.

Figure S3 Effects of PASD1 on INF-γ-induced STAT1 phosphorylation.

(**A**) Overexpression of PASD1 has no marked effects on INF-γ-induced STAT1 phosphorylation. HEK293 cells (2×10^5) were transfected with empty vector or PASD1 expression plasmid (0.4 μg). Twenty hours after transfection, cells were treated with IFN-γ (100 ng/mL) for the indicated times before immunoblot experiments were performed.

(**B**) Knockdown of PASD1 enhances INF-γ-induced STAT1 phosphorylation.

The control and PASD1-RNAi stable cells (2×10^5) were seeded in a 12-well plate and treated with IFN-γ (100 ng/mL) for the indicated times before immunoblot experiments were performed.

Figure S4 Effects of PASD1 on STAT3-mediated transcription of pro-oncogenic genes.

(**A**) Effects of PASD1 overexpression on STAT3-mediated transcription of pro-oncogenic genes. Total RNAs of the control and PASD1-overexpressed cells (2×10^5) were extracted, reverse-transcribed and subjected to qPCR analysis to measure the transcription of the indicated genes. Results are represented as mean ± SD, n=3, *$p < 0.05$, **$p < 0.01$,***$p<0.001$.

(**B**) Effects of PASD1 knockdown on STAT3-mediated transcription of pro-oncogenic genes. Total RNAs of the GFP-RNAi control and PASD1-RNAi cells (2×10^5) were extracted, reverse-transcribed and subjected to qPCR analysis to measure the transcription of the indicated genes. Results are represented as mean ± SD, n=3, *p < 0.05, **p < 0.01,***p<0.001.

Table S1. Specific sequences of primers used in RT-PCT experiments.

Gene	Forward sequence	Reverse sequence
BCLXL	5'-GCCACTTACCTGAATGACCACC-3'	5'-AACCAGCGGTTGAAGCGTTCCT-3'
BIRC5	5'-CCACTGAGAACGAGCCAGACTT-3'	5'-GTATTACAGGCGTAAGCCACCG-3'
CCND1	5'-TCTACACCGACAACTCCATCCG-3'	5'-TCTGGCATTTTGGAGAGGAAGTG-3'
C-FOS	5'-GCCTCTCTTACTACCACTCACC-3'	5'-AGATGGCAGTGACCGTGGGAAT-3'
GAPDH	5'-CGGAGTCAACGGATTTGGTCG-3'	5'-AGCCTTCTCCATGGTGGTGAAG-3'
ICAM1	5'-TCAGTGTGACCGCAGAGGACGA-3'	5'-TTGGGCGCCGGAAAGCTGTAGAT-3'
IL-6	5'-TTCTCCACAAGCGCCTTCGGTC-3'	5'-TCTGTGTGGGGCGGCTACATCT-3'
IRF1	5'-GAGGAGGTGAAAGACCAGAGCA-3'	5'-TAGCATCTCGGCTGGACTTCGA-3'
MCL1	5'-CCAAGAAAGCTGCATCGAACCAT-3'	5'-CAGCACATTCCTGATGCCACCT-3'
MMP2	5'-AGCGAGTGGATGCCGCCTTTAA-3'	5'-CATTCCAGGCATCTGCGATGAG-3'
MMP9	5'-GCCACTACTGTGCCTTTGAGTC-3'	5'-CCCTCAGAGAATCGCCAGTACT-3'
PASD1	5'-GAAGAGAGGACTTGGTTGCTGC-3'	5'-GGAGATCAGGAATGACAACGTGG-3'
SOCS1	5'-TTCGCCCTTAGCGTGAAGATGG-3'	5'-TAGTGCTCCAGCAGCTCGAAGA-3'
SOCS3	5'-CATCTCTGTCGGAAGACCGTCA-3'	5'-GCATCGTACTGGTCCAGGAACT-3'
STAT1	5'-ATGGCAGTCTGGCGGCTGAATT-3'	5'-CCAAACCAGGCTGGCACAATTG-3'
VEGF	5'-TTGCCTTGCTGCTCTACCTCCA-3'	5'-GATGGCAGTAGCTGCGCTGATA-3'

Supplementary Information

Figure S1

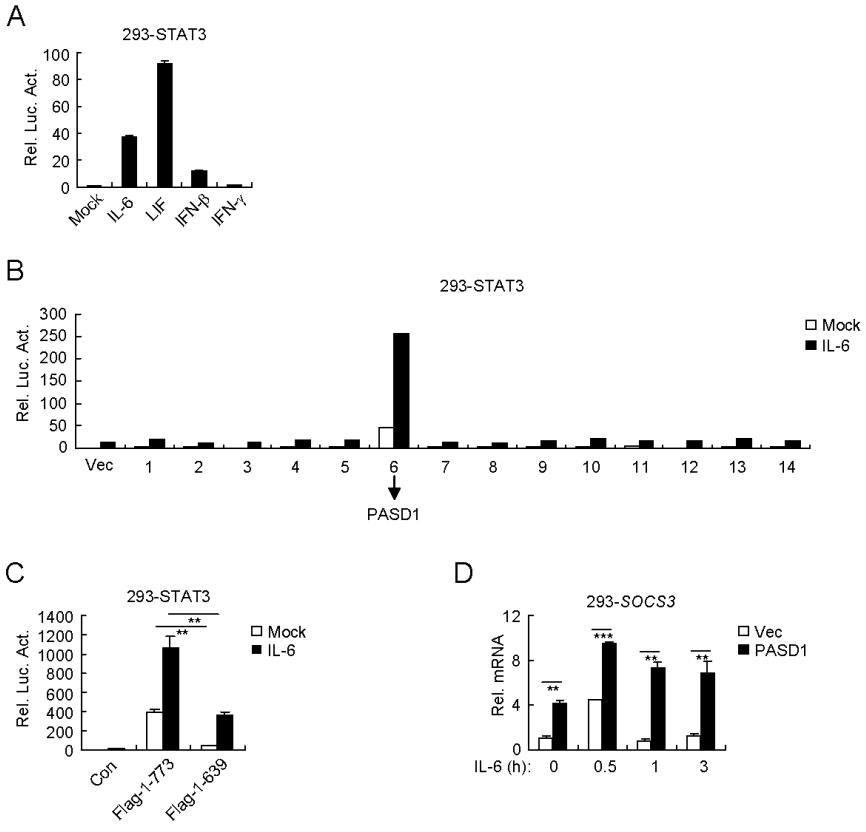

Figure S2

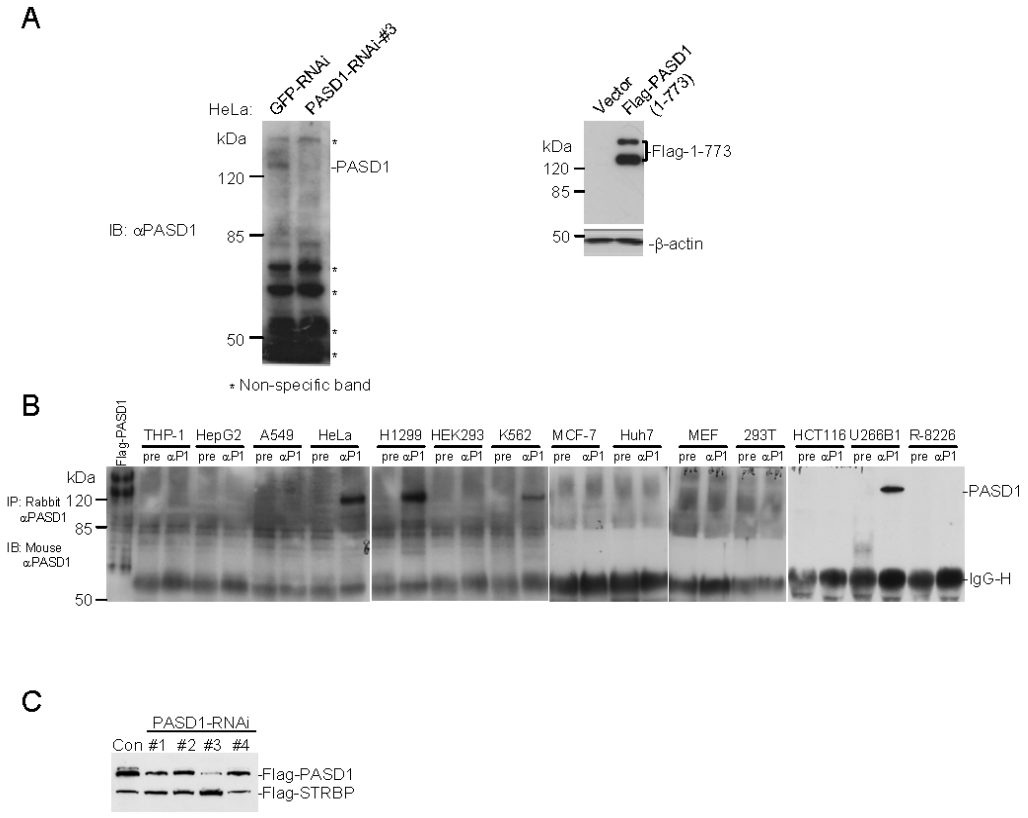

Figure S3

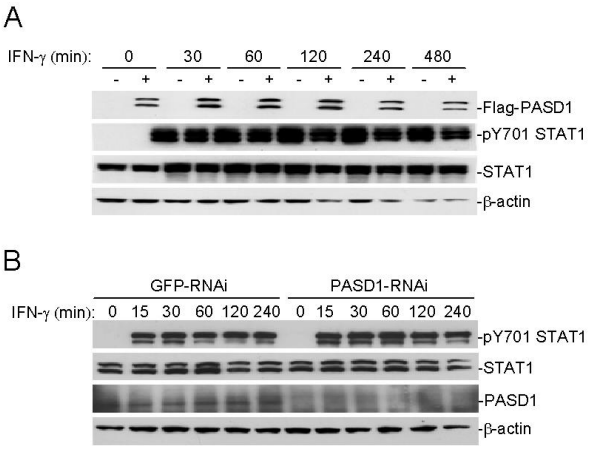

Figure S4