Supplementary Figure S1  The time course of MST1 activity and Daxx abundance in BV-2 cells after IFN-γ treatment. BV-2 cells were treated with IFN-γ (100 U/ml) for the indicated times. Cell lysates were subjected to immunoprecipitation with anti-MST1 antibody, and the resulting precipitates were examined for MST1 activity by immune complex kinase assay with histone H2B as substrate. The relative kinase activity is shown below each lane. Cell lysates were also examined by immunoblot analysis (IB) with antibodies to Daxx or to MST1.
**Supplementary Figure S2** Effect of AG490 on IFN-γ–induced increase in Daxx abundance and MST1 activity in BV-2 cells. BV-2 cells were left untreated or treated with 10 mM AG490 for 2 h and then to IFN-γ (100 U/ml) for 16 h as indicated. Cell lysates were subjected to immunoprecipitation with anti-MST1 antibody, and the resulting precipitates were examined for MST1 activity by immune complex kinase assay with histone H2B as substrate. Cell lysates were also examined by immunoblot analysis with antibodies to Daxx or to MST1.
**Supplementary Figure S3** MST1 does not phosphorylate Daxx *in vitro*. BV-2 cells were left untreated or treated with IFN-γ (100 U/ml) for 16 h, and then lysed. Cell lysates were subjected to immunoprecipitation with anti-MST1 antibody or rabbit preimmune IgG. The resulting immunoprecipitates were examined for a kinase activity with the use of histone H2B (1 μg) or His_{6}-tagged Daxx (1 μg) as substrate. Substrate proteins (1 μg) were also subjected to SDS-PAGE on 10% gel and stained with Coommasie Blue solution.
**Supplementary Figure S4** Effect of siRNA-mediated Daxx depletion on IFN-γ-induced MST1 activation in BV-2 cells. *Left*, BV-2 cells were stably transfected with pSuper-retro vectors for GFP control siRNA or mouse Daxx siRNA, yielding BV-2/si-Control or BV-2/si-Daxx cells, respectively. After BV-2/si-Control or BV-2/si-Daxx cells were incubated with IFN-γ (100 U/ml) for 16 h, cell lysates were subjected to immunoprecipitation with anti-MST1 antibody and the resulting precipitates were assayed for MST1 activity by immune complex kinase assay. Cell lysates were also examined directly by immunoblot analysis with antibodies to Daxx and MST1. *Right*, the intensity of the bands corresponding to histone H2B phosphorylation was quantified in three independent experiments by densitometry.
Supplementary Figure S5 MST1 binds to Daxx(1-500), but not to Daxx(498-740). 293T cells were transfected for 48 h with a vector encoding Flag-MST1 together with vectors for HA-tagged Daxx variants, as indicated. Cell lysates were subjected to immunoprecipitation with anti-Flag antibody, and the resulting precipitates were examined by immunoblot analysis with anti-HA antibody. Cell lysates were also examined directly by immunoblot analysis with antibodies to Flag or to HA.
Supplementary Figure S6 Effect of WW45 or Rassf1a on Daxx-mediated MST1 activation in MEFs. Wild-type MEF and MEF_{WW45^{+/+}} (A) or MEF_{Rassf1a^{+/+}} (B) cells were transfected for 48 h with a vector encoding Flag-MST1 alone or together with HA-Daxx. Cell lysates were assayed for MST1 activity by immune complex kinase assay with histone H2B as substrate. Cell lysates were also examined by immunoblotting with antibodies to HA, to Flag, or to WW45 (A) or to Rassf1a (B).
Supplementary Figure S7 Effect of siRNA-mediated Rassf5 depletion on the IFN-γ–induced MST1 activation in BV-2 cells. BV-2 cells were transfected with either GFP or Rassf5 siRNA oligonucleotides for 48 h, incubated further for 16 h in the absence or presence of IFN-γ (100 U/ml), and then lysed. Cell lysates were subjected to immunoprecipitation with anti-MST1 antibody and the resulting precipitates were assayed for MST1 activity by immune complex kinase assay. Cell lysates were also examined directly by immunoblot analysis with antibodies to Daxx, to MST1, or to Rassf5. The siRNA duplexes specific for mouse Rassf5 mRNA were synthesized chemically by Invitrogen and targeted to the sequences 5’-GAAGATTGACAGCTATAACAGC -3’ (Yuan et al, 2009).

Supplementary Figure S8 PML does not interact with MST1 in BV-2 cells. (A) BV-2 cells were left untreated or treated with IFN-γ (100 U/ml) for 16 h. Cell lysates were subjected to immunoprecipitation (IP) with either mouse preimmune IgG or anti-PML antibody (α-PML, mouse monoclonal, Upstate), and the resulting precipitates were subjected to immunoblot analysis with antibodies to Daxx or to MST1. Cell lysates were also directly immunoblotted with antibodies to Daxx, to MST1, or to PML. (B) BV2 cells were transfected for 24 h with siRNA oligonucleotides targeting GFP (control) or PML mRNA and were then incubated for 16 h in the absence or presence of IFN-γ (100 U/ml). Cell lysates were subjected to immunoblot analysis with antibodies to phospho-MST1, to Daxx, to PML, or to MST1. The siRNA duplexes specific for mouse PML mRNAs were synthesized chemically by Invitrogen and targeted to the sequences 5’-GCGCAAGTCCAATATCTTC-3’ (Park et al, 2007).

Supplementary Figure S9 Homodimerization of Daxx. Daxx⁻/⁻ MEFs were transfected for 48 h with the indicated combinations of plasmids for Flag- or HA-tagged Daxx variants, after which cell lysates were subjected to immunoprecipitation with anti-HA antibody and the resulting precipitates were subjected to immunoblotting with anti-Flag antibody. Cell lysates were also examined directly by immunoblot analysis with antibodies to Flag or to HA.
Supplementary Figure S10 Effect of IFN-γ on MST1 activity and apoptosis in primary mouse microglia and astrocytes. (A) Primary mouse microglia and astrocyte were left untreated or treated with IFN-γ (100 U/ml) for 16 h. Cell lysates were subjected to immunoprecipitation with anti-MST1 antibody, and the resulting precipitates were examined for MST1 activity by immune complex kinase assay with histone H2B as substrate. The relative kinase activity is shown below each lane. Cell lysates were also examined directly by immunoblot analysis with antibodies to Daxx, to MST1, or to α-tubulin. (B) Primary mouse microglia and astrocytes were left untreated or treated with IFN-γ (100 U/ml) for 48 h. Where indicated, microglia were pretreated with 50 μM z-VAD-fmk for 2 h prior to the IFN-γ treatment. Cells were then analyzed for apoptotic cell death with the TUNEL method (Roche applied science, In Situ Cell Death Detection Kit, TMR red). Data are the mean of triplicate determinations ± SD in one representative of three independent experiments.
Supplementary Figure S11 Immunohistochemical images to confirm the expression of Daxx in microglia in the cerebral cortex after IFN-γ injection. WT mice were subjected to stereotaxic injection of IFN-γ or PBS into the cerebral cortex. Brain sections adjacent to the injection sites were prepared and subjected to immunohistochemical staining with antibodies to Daxx and to Iba-1 at one day after IFN-γ or PBS injection, as described in the Materials and Methods. The images were visualized with Olympus BX51 fluorescence microscope equipped with Olympus DP70 digital camera. Scale bar, 100 µm.
Supplementary Figure S12 Depletion of MST1 expression by RNAi does not affect NO production in BV-2 cells. BV-2/si-Control and BV-2/si-MST1 cells in 6-well culture dishes (1x10^6 cells in 2 ml/well) were incubated in the absence or presence of IFN-γ (100 U/ml) for 24 h. Culture medium (50 μl) from each well was assayed for NO release (nitrite formation) by Griess method (Sigma). Data are the mean of triplicate determinations ± SD.
Supplementary Figure S13 Effects of SP600125 and SB203580 on IFN-γ–induced apoptosis in BV-2 cells. BV2 cells were left untreated or treated with IFN-γ (100 U/ml) for 48 h. Where indicated, cells were pretreated with 10 μM JNK inhibitor (SP600125) or 20 μM p38 inhibitor (SB203580) for 2 h prior to the IFN-γ treatment. Cells were then analyzed for apoptotic cell death with the TUNEL method. Data are the mean of triplicate determinations ± SD.
**Supplementary Figure S14** Effect of IFN-γ on Daxx abundance and MST1 activity in primary alveolar macrophages. Rat alveolar macrophages were isolated from 6-wk-old male Sprague–Dawley rats and cultivated as described previously (Park et al, 2000). The cells were left untreated or treated with IFN-γ (100 U/ml) for 16 h and lysed. (A) Cell lysates were immunoblotted with antibodies to Daxx or to actin. (B) Cell lysates were subjected to immunoprecipitation with anti-MST1 antibody and the resulting precipitates were assayed for MST1 activity by immune complex kinase assay. Cell lysates were also examined directly by immunoblot analysis with antibodies to MST1.