PNAS
www.pnas.org

Supplementary Information for

KDM3A Histone Demethylase Functions as an Essential Factor for Activation of JAK2-STAT3 Signaling Pathway

Hyunkyung Kim[a,1], Dongha Kim[a,1], Seon Ah Choi[a], Chang Rok Kim[a], Se Kyu Oh[a], Ki Eun Pyo[a], Joomyung Kim[a], Seung-Hoon Lee[b], Jong-Bok Yoon[b], Yi Zhang[c], and Sung Hee Baek[a,*]

[a]*Creative Research Initiatives Center for Epigenetic Code and Diseases, Department of Biological Sciences, Seoul National University, Seoul 08826, South Korea*

[b]*Department of Biochemistry, Translational Research Center for Protein Function Control, Yonsei University, Seoul 03722, South Korea*

[c]*Howard Hughes Medical Institute, Harvard Medical School, Boston, MA 02115, USA*

[1]*These authors contributed equally to this work*

Sung Hee Baek

Email: sbaek@snu.ac.kr

www.pnas.org/cgi/doi/10.1073/pnas.1805662115

SI Materials and Methods

Reporter assay

Using a luciferase system (Promega), the *M67* (4X-STAT response element)-luciferase activity was measured using a luminometer at 48 hr after transfection and normalized by β-galactosidase activity. Values are expressed as mean ± s.d. of at least three independent experiments.

Quantitative RT-PCR

For qRT-PCR analyses, total RNAs were extracted using Trizol (Invitrogen) and reverse transcribed using RevertAid™ Reverse Transcriptase (Enzynomics). The following primers were used for the PCR reactions:

hMYC: forward 5'-CAGCTGCTTAGACGCTGGATT-3',

reverse 5'-GTAGAAATACGGCTGCACCGA-3';

hHPRT; forward 5'-TGACACTGGCAAAACAATGCA-3',

reverse 5'-GGTCCTTTTCACCAGCAAGCT-3';

The abundance of mRNA was detected by an ABI prism 7300 system with SYBR Green (Molecular Probes).

ChIP assays

The following ChIP primers for STAT binding element of *hMYC* were used for the PCR reactions:

forward 5'-GCCTCTGGCCCAGCCCTCCCGCTGAT-3',

reverse 5'-GCAAAGTGCCCGCCC GCTGCTATGG-3'.

Identification of phosphorylation sites by LC-MS/MS

Tryptic digests of the KDM3A were analyzed by nanoelectrospray LC-MS/MS. High pressure liquid chromatography separation was performed on an Ultimate instrument equipped with Famous Autosampler (LC Packings). The columns were constructed from fused silica capillary tubes with outer diameters of 360 μm and inner diameters of 75 μm and packed to a length of 15 cm with 300-Å C18 beads with a 5 μm diameters (Grace Vydac). The outlet of the nanocolumn was connected in-line to a distal coated silica PicoTip needle (New Objective). The solvent system consisted of solvent A (0.1 % formic acid and 5 % acetonitrile) and solvent B (0.1 % formic acid and 90 % acetonitrile). The gradient was linear from 0 to 40 % solvent B over 50 min and then 90 % solvent B for 5 min. Tandem mass spectra were recorded on an API QSTAR Pulsar Q-TOF mass spectrometer (Applied Biosystems) in the information-dependent acquisition mode. The MS/MS spectra were used to search the National Center for Biotechnology Information nonredundant and expressed sequence tag databases using Mascot (Matrix Science). Identified phosphopeptides were manually verified.

Immunocytochemistry

HeLa cells were grown on coverslips and washed with phosphate-buffered saline (PBS). Cells were fixed with 2 % paraformaldehyde in PBS for 10 min at room temperature, and permeabilized with 0.5 % Triton X-100 in PBS. After blocking, anti-Flag, anti-JAK2 and anti-H3K9me2 antibodies were labeled by Alexa Fluor 594 and 488 mouse and rabbit IgG Labeling Kits, respectively (Molecular Probe).

In vitro cell motility assay

Wound healing scratching motility assay was performed in HeLa cells transfected with pLKO.1 control vector or pLKO.1-shKDM3A. Cells were seeded in 6-well culture plates and cultured until they reached confluence. Cells were scratched with a 200 μl micro-pipette tip and incubated at 37℃ for 48 hr. Photomicrographs of the closed gap were captured at 0 hr, 24 hr, and 48 hr using an EVOS xl transmitted light microscope (AMG, Bothell, WA). Migration distance of the cells was quantified by distance of gap using graph prism software. Values are expressed as means ± s.e.m.

Cell proliferation assay

pLKO.1 control vector or pLKO.1-sh*KDM3A* was transfected into HeLa cells by lipofectamine reagents in the absence or presence of IL-6. One day after transfection, cells were detached and subcultured on 24-well plates. Cell proliferation was counted for each 6 hr using JuLi^{TM} stage live imaging instrument (NanoEnTek Inc,) and analyzed with JuLi^{TM} STAT software according to manufacturer's guide.