**Supplementary Information**

**Figure Legends**

***Fig. S1 Effects of other oxidants on GAPDH aggregation in vitro and detection of cellular disulfide-bonded aggregations of GAPDH in HeLa cells treated with NOC18***

(A) Effects of other oxidative stresses, such as $H_2O_2$, S-nitrosoglutathione (GSNO), and peroxynitrite (ONOO-) at a concentration of 100 μM on the turbidity of solutions containing purified WT-GAPDH are shown. (B) Concentration-dependent effects of dopamine on the turbidity of solutions containing purified WT-GAPDH are shown. Treatment procedures were the same as for NOR3. Data are the mean ± S.D. of three samples. (C) The transfection efficiency of WT- or C152A-GAPDH cDNA is shown. Total cell lysates (10 μg) of tranfected HeLa cells were immunoblotted with an anti-GAPDH monoclonal antibody (upper panel) and an anti-myc monoclonal antibody (lower panel). (D) Effects of 1 mM 1-hydroxy-2-oxo-3,3-bis(2-aminoethyl)-1-triazene (NOC18), (Dojindo, Kumamoto, Japan), a nitric oxide donor, on the amount of myc-tagged GAPDH in the particulate fractions (5 μg/lane) assessed by Western blotting with an anti-myc monoclonal antibody are shown. Non-reduced (left panel) or reduced (right panel) 5-20% SDS-PAGE was performed. An anti-histone H2B polyclonal antibody was used as a loading control (left lower panel).

***Fig. S2 Dopamine caused oxidative stress in SH-SY5Y cells***

SH-SY5Y cells were treated without or with dopamine (300 μM) for 4 h and stained with CM-H2DCFDA (10 μM for 15 min) (1). Reactive oxygen species (ROS) formation (green) in the cells were detected by fluorescence microscopy. DIC; differential interference contrast**.**

***Fig. S3 Detection of intracellular GAPDH aggregate formation***

An analytical method was developed to evaluate intracellular aggregates automatically. First, raw data captured by confocal microscopy was grayed and inverted by Photoshop ver. 6.0 (Adobe). Next, the converted images were transformed to a “binary” option using Scion image software, and the threshold (T) for the background of control cells (left panels) was set at T=100. The “outline" option was applied to resulting images (right panel) to detect particles (arrows). The distribution of these imaged particles closely match the strongly stained material derived from intracellular aggregates. Thus, cells with more than two particles were defined as aggregate-positive, and those with one or no particles were scored as negative.

***Fig. S4 Characteristics of DOX-inducible, myc-tagged WT- or C152A-GAPDH expressing SH-SY5Y cells.***

(A) Time-courses of expression of DOX-induced, myc-tagged GAPDH (WT and C152A) in stable SH-SY5Y cells assessed by Western blotting with an anti-GAPDH polyclonal antibody are shown. (B) Immmunofluorescences of DOX-inducible, GAPDH-expressing SH-SY5Y cells with (+) or without (-) DOX-treatment are shown. Six days after DOX-treatment, myc-tagged WT- or C152A-GAPDH (green) was detected by an anti-myc monoclonal antibody, whereas no signal was detected in the absence of DOX. Nuclei were stained by DAPI (blue). Scale bar=200 μm. (C) After cells were treated with DOX (1 μg/mL) for 6 days, lysates extracted from mock-, WT- or C152A-expressing cells were subjected to a GAPDH enzymatic assay. Data are indicated as the mean ± S.D. of four samples

***Fig. S5 Characteristics of GAPDH-Tg mice***

(A) The GAPDH transgenic construct is shown. (B) The protein level and activity of GAPDH in the hippocampus of GAPDH-Tg or littermate control mice are shown. Data are the mean ± S.D. of eight samples (*t* test, *, $P<0.05$ vs. WT). (C) The levels of GAPDH mRNA in the hippocampus GAPDH-Tg or littermate control mice are shown. Data are the mean ± S.D. of eight samples (*t* test, *, $P<0.05$ vs. WT).

## Materials and methods

### *Chemicals and antibodies*

Unless otherwise noted, chemicals were of analytical grade. For generation of GAPDH transgenic mice, pSI-GAPDH cDNA (2) was cloned into pBacMam2 (NovaGen) using the *Sal* I-*Not* I sites, and the insert was digested at unique *Sph* I and *Hind* III sites.

### *Cellular GAPDH enzymatic assay*

Cells were washed once with PBS (-), scraped, and sonicated in a buffer containing 50 mM Tris-HCl (pH 7.5), 50 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 1 mM EDTA, 0.25 M sucrose, and protease inhibitor cocktail (Nacalai tesque, Kyoto, Japan). The homogenates were centrifuged (21,000 × *g* for 10 min at 4°C) and the cleared lysates (1-2 μg) were used in an *in vitro* enzymatic assay as noted above. Protein concentrations of the samples were measured by a Bradford assay (Bio-Rad).

### *Detection of reactive oxygen species (ROS)*

Cells treated without or with dopamine (300 μM, 4 h) were washed with PBS (-) twice and incubated with 10 μM CM-$H_2$DCFDA (Molecular Probes) at 37°C for 15 min (1). Cells

were imaged under a Nikon fluorescence microscope (TE-2000) with a CCD camera. Fluorescent images were acquired with a single 500 ms exposure, followed by a DIC image of the same field.

***Generation of GAPDH transgenic mice***

Full-length rat GAPDH was placed under the control of the chicken β-actin promoter in a modified pCAGGS vector (3), pBacMam2 vector (NovaGen). The insert was manually injected into oocytes of C57BL/6 mice. Integration of the transgene into the mouse genome was confirmed by genomic PCR with the transgene specific primers, 5'-CTGGGCAACGTGCTGGTTATTGTG-3' (upstream), and 5'-CATCACAAACATGGGGGCATCAGC-3' (downstream). Three lines (#4, #20, and #41) were established by mating with C57BL/6 mice to maintain the purity of the genetic background. Line #41 was used in the present study.

***Real time RT-PCR***

Total RNA from mouse hippocampus was prepared using the RNeasy kit (Quiagen). cDNA was synthesized by an Omniscript reverse transcriptase (Qiagen). The sequences of the oligonucleotide primers for PCR were as follows: GAPDH, 5'-TGCTGAGTATGTCGTGGAGTCT-3' (upstream), and 5'-AATGGGAGTTGCTGTTGAAGTC-3' (downstream); 18S-rRNA, 5'-GTAACCCGTTGAACCCCATT-3' (upstream), and 5'-CCATCCAATCGGTAGTAGCG-3' (downstream). PCR cycling conditions were as follows: DNA denaturation at 94°C for 30 s, primer annealing at 60°C for 1 min, and DNA extension at 72°C for 1 min (22 cycles). Semi-quantification of GAPDH and 18S-rRNA mRNA levels was performed using SYBR Green Realtime PCR Master Mix plus (TOYOBO) and LineGene (BioFlux).

## References


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**Fig. S1**

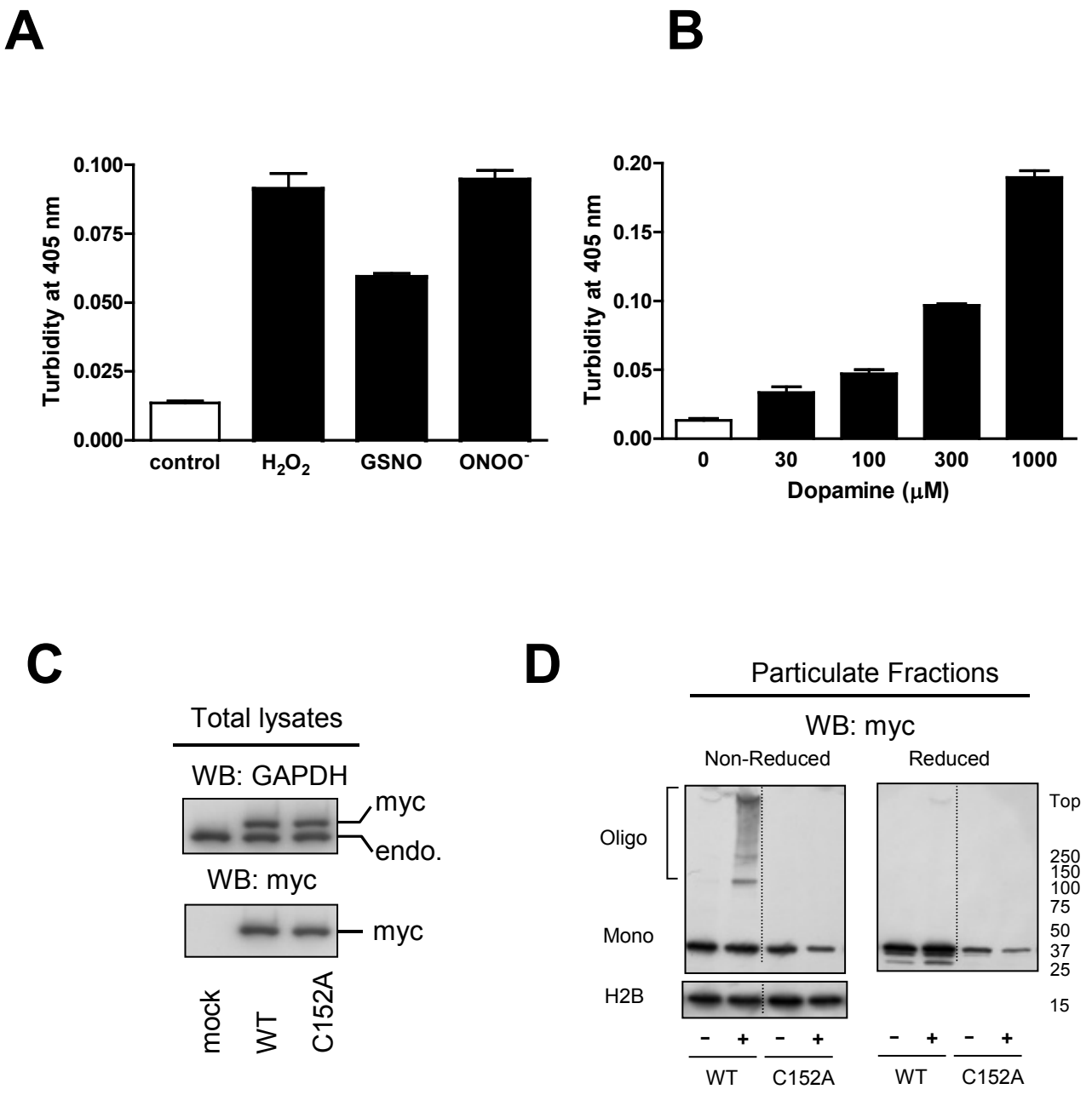

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**Fig. S2**

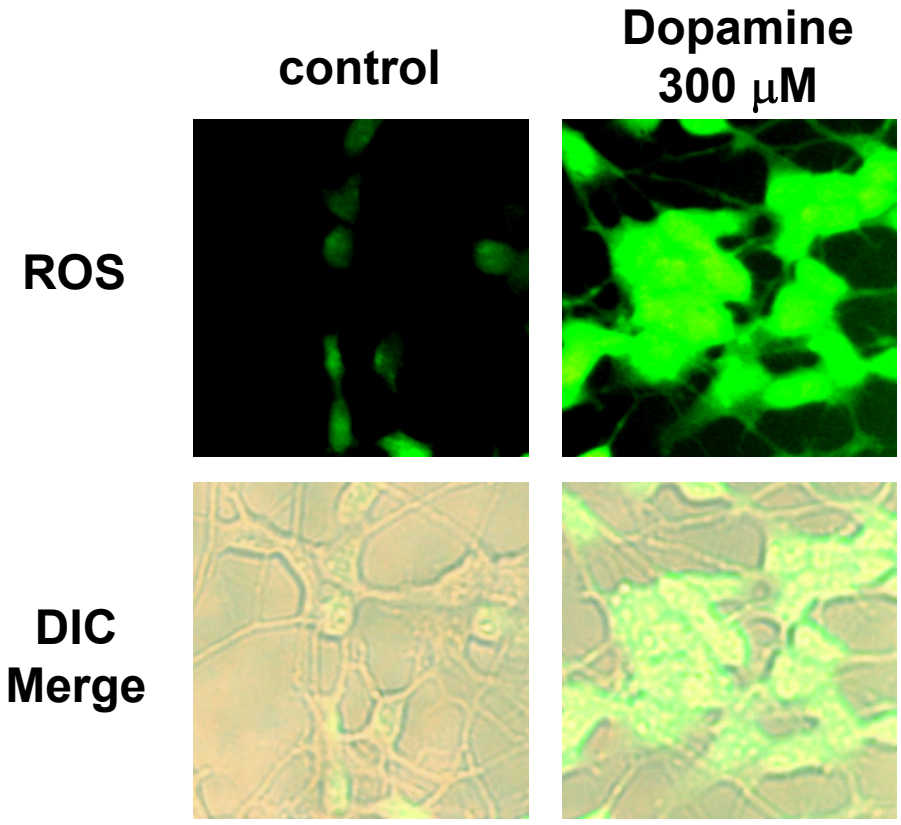

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**Fig. S3**

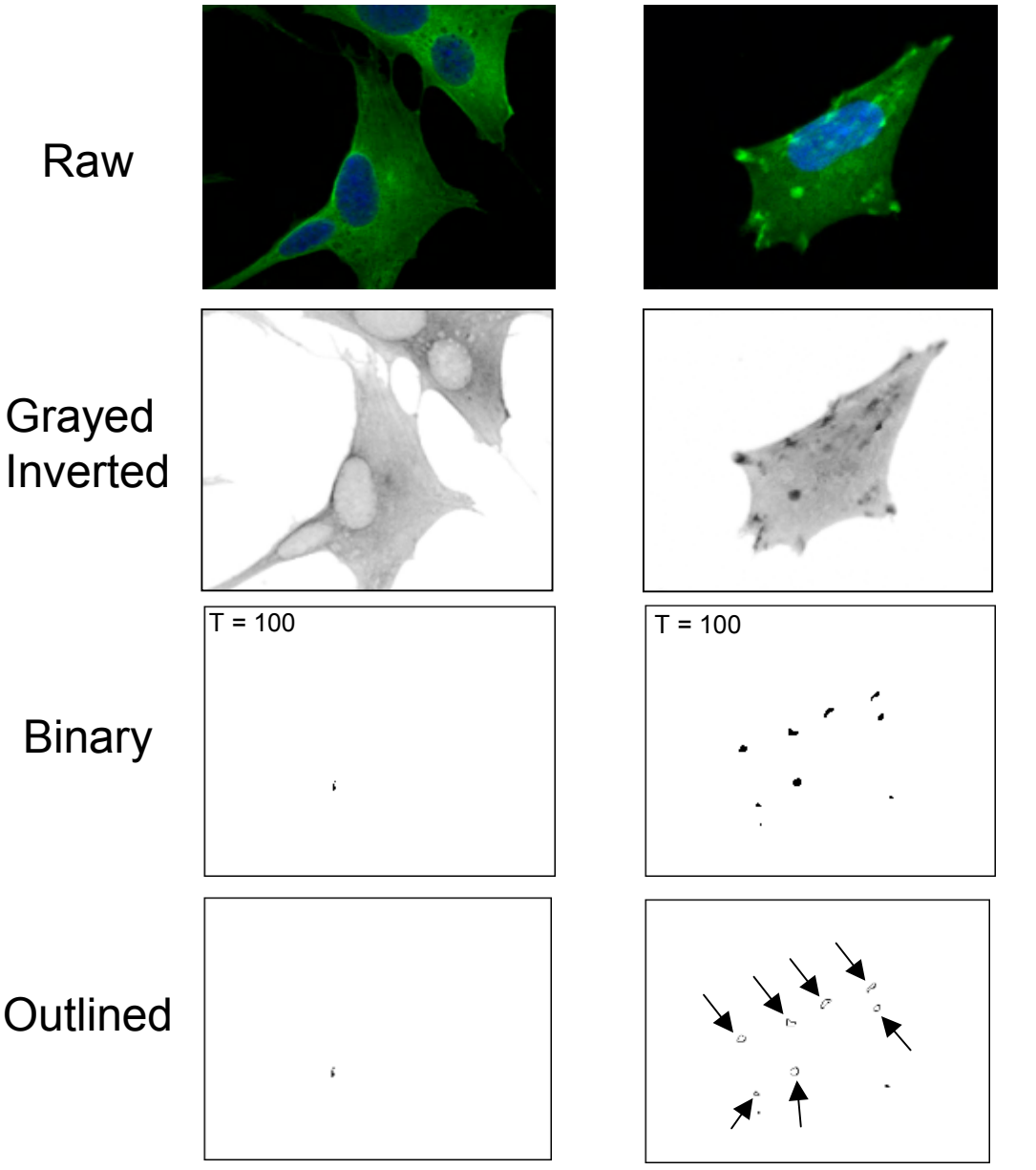

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**Fig. S4**

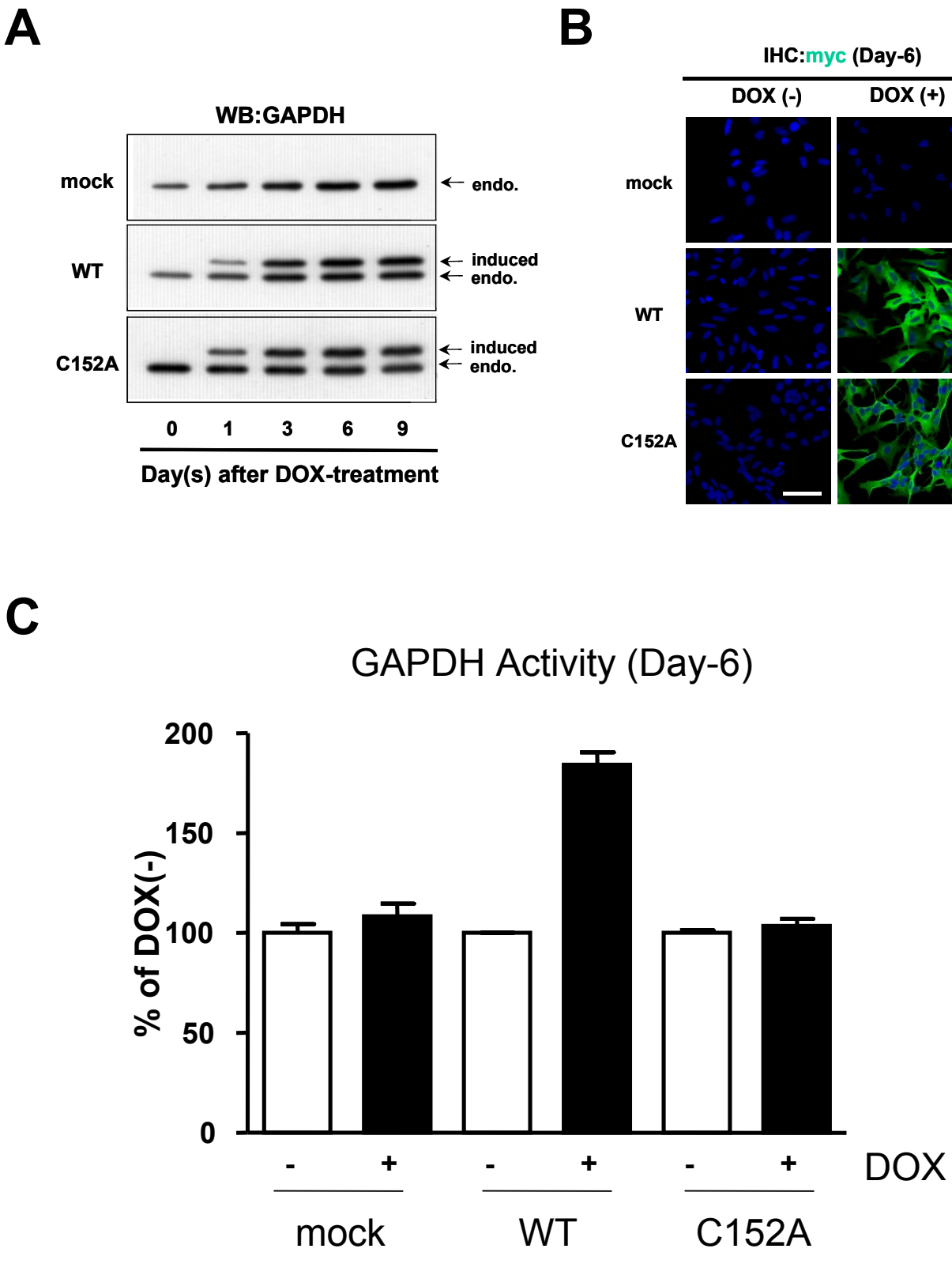

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**Fig. S5**