Supplementary Figure Legends

SUPPLEMENTARY FIGURE 1**. Quantitative analysis of MT density in cultured cardiac myocytes.**
Cultured cardiac myocytes were treated with LIF (10 ng/ml, 24 h) or 0.1% (w/v) BSA in PBS as a control (Con). The myocytes were then fixed, immunostained for glu-tubulin. Staining with phalloidin-TRITC revealed contractile actin filaments. Regions of interest (ROI) were delineated as individual cardiac myocytes as described in the Materials and Methods. Pixel density and cell area (μm^2) were then quantified to determine glu-MT density (pixels/μm^2). A minimum of 100 cells was measured from 10 randomly selected fields. These images are representative of randomly selected fields of cardiac myocytes used for quantitative analysis of MT density. Microtubule densities representative of three independent experiments (n=3) were expressed as an average ± standard error of the mean (SEM). Scale bar = 50 μm.

SUPPLEMENTARY FIGURE 2**. Temporal analysis of glu-tubulin and phosphorylated STAT3 changes in TnI-203/MHC-403 mice.**
A, Protein extracts from the hearts of 5, 10, 14 and 18 day old non-transgenic (NTG) and double-mutant TnI-203/MHC-403 mice (n=3) were immunoblotted for glu-tubulin and Y705 phosphorylated STAT3. *B,* Glu-tubulin and pY705-STAT3 bands were quantified by densitometric analysis and expressed as fold-increases over levels measured in 5 day old samples (mean±SD, n=3).

SUPPLEMENTARY FIGURE 3**. Hypertrophic agonist stimulated increases in cardiac myocyte size.**
Cardiac myocytes cultured on laminin-coated glass coverslips were stimulated for 24 h with PE (100 μM), LIF (10 ng/ml), OSM (10 ng/ml), or 0.1% (w/v) BSA in PBS as a non-stimulated control (Con) before fixing and immunofluorescent staining. Cell size (μm^2) was then determined as part of image analysis of MT density as described in "Experimental Procedures". Cell size values are expressed as mean±SE from three independent experiments. '*' denotes statistical difference ($p<0.05$) compared to Con sample.

SUPPLEMENTARY FIGURE 4**. Short-term hypertrophic agonist treatment does not increase glu-tubulin levels in cardiac myocytes.**
Cultured cardiac myocytes were treated (0 - 8 h) with PE (100 μM) (*A*), LIF (10 ng/ml) (*B*), or OSM (10 ng/ml) (*C*). Protein lysates were prepared and immunoblotted for glu-tubulin and α-tubulin.

SUPPLEMENTARY FIGURE 5**. Nocodazole-resistant MTs in cardiac myocytes treated with PE.**
Con or PE-stimulated (100 μM) cardiac myocytes were treated with nocodazole (NZ, 2 μM, 1 h) before fixing and immunostaining for α-tubulin to reveal the stabilized drug-resistant MT network. Scale bar = 50 μm.

SUPPLEMENTARY FIGURE 6**. Hypertrophic agonists, PE, LIF and OSM, stimulate different STAT3 and ERK phosphorylation kinetics.**
Cultured cardiac myocytes were treated (0 - 8 h) with PE (100 μM) (*A*), LIF (10 ng/ml) (*B*), or OSM (10 ng/ml) (*C).* Protein lysates were prepared and immunoblotted for active, phosphorylated STAT3 and ERK using phospho-specific antibodies (pY-STAT3 and pT/pY-ERK). Total STAT3 and ERK levels were confirmed to be equivalent in the samples. *D,*

Cultured cardiac myocytes were treated for 24 h with PE (100 μM), LIF (10 ng/ml), OSM (10 ng/ml) or 0.1% (w/v) BSA in PBS as a control (Con) before STAT3 and ERK phosphorylation and protein levels were determined by immunoblot analysis.

SUPPLEMENTARY FIGURE 7**. Inhibition of STAT3 and ERK activation by pharmacological inhibitors or RNAi knockdown .**
Cultured cardiac myocytes were pretreated with U0126 (10 μM, 30 min) (*A*), AG490 (50 μM, 30 min) (*B*), Stattic (2 μM, 30 min) (*C*), or a vehicle control (0.1% [v/v] DMSO). Subsequently, myocytes were stimulated (15 min) with PE (100 μM), LIF (10 ng/ml), OSM (10 ng/ml) or 0.1% (w/v) BSA in PBS as a control (Con). Protein lysates were prepared and immunoblotted for pY-STAT3, STAT3, pT/pY-ERK and ERK levels. *D*, Cardiac myocytes were transfected with STAT3 siRNA target 3 or non-targeting control siRNA (Con siRNA) before stimulation with LIF (10 ng/ml, 15 min) or OSM (10 ng/ml, 15 min). STAT3 and ERK phosphorylation and protein levels were then determined by immunoblot analysis. Identical results were obtained with STAT3 siRNA target 1 and STAT3 siRNA target 2 (data not shown).

SUPPLEMENTARY FIGURE 8**.** Representative images of glu-tubulin immunostaining in PE- and/or U0126-treated cardiac myocytes. Scale bar = 50 μm.

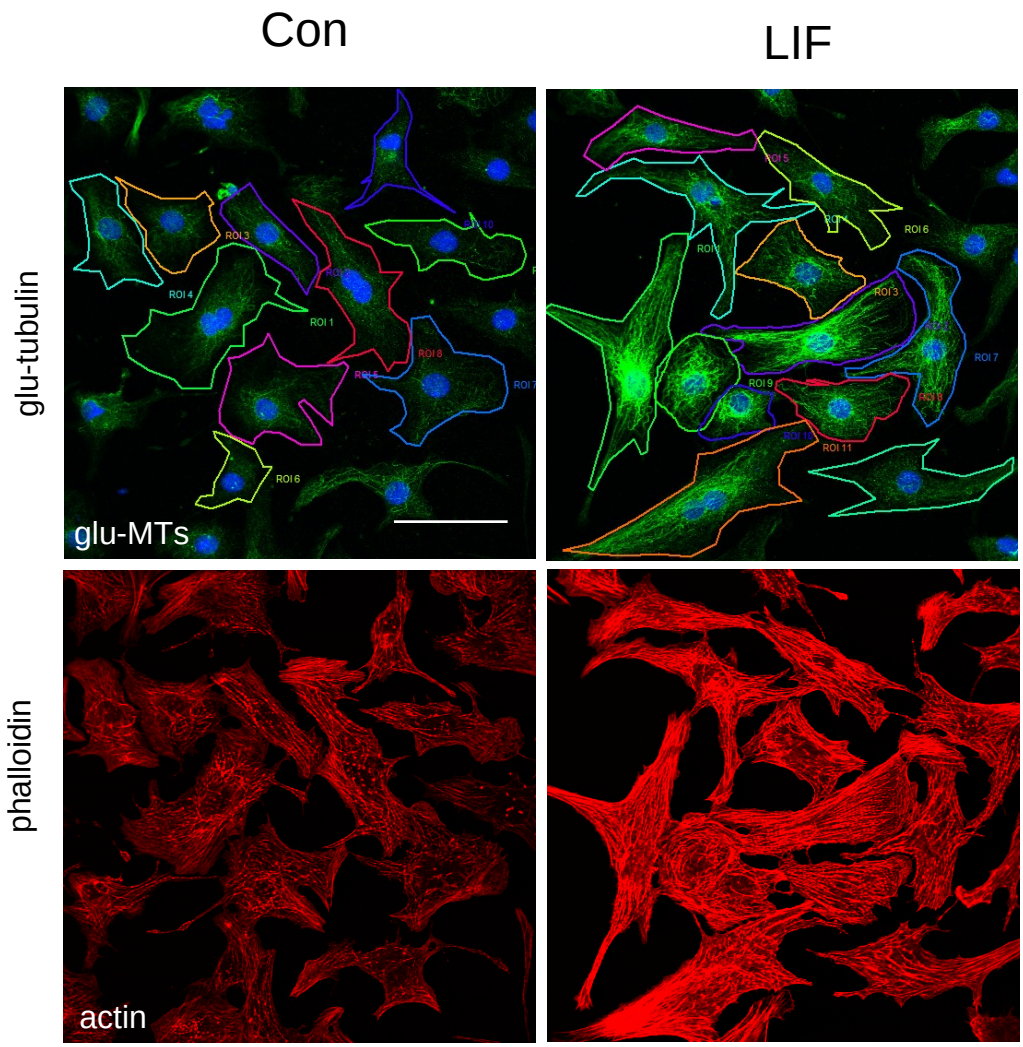
SUPPLEMENTARY FIGURE 9**.** Representative images of glu-tubulin immunostaining in AG490- or DMSO-treated cardiac myocytes subsequently exposed to LIF or OSM. Scale bar = 50 μm.

SUPPLEMENTARY FIGURE 10**.** Representative images of glu-tubulin immunostaining in Stattic- or DMSO-treated cardiac myocytes subsequently exposed to LIF or OSM. Scale bar = 50 μm.

	Sham (n=6)	Compensated Hypertrophy (n=7)	Decompensated Hypertrophy (n=8)
BW, g	27.3±0.3	26.2±0.4	26.4±0.6
IVS, mm	0.71±0.04	1.02±0.07	1.03±0.04
LVPW, mm	0.72±0.03	1.09±0.09*	1.03±0.04
EDD, mm	3.50±0.06	3.23±0.15	3.71±0.15
ESD, mm	1.61±0.05	1.49±0.15	2.36±0.18*
FS, %	54±1	54±4	37±3*
EF, %	90±1	89±2	74±4*
HR, bpm	584±32	578±13	532±8
AoPg, mm Hg#	4±1	25±1*	29±3*
HW/BW, mg/g	4.01±0.15	5.69±0.23*	6.71±0.32*
LW/BW, mg/g	4.81±0.10	7.31±0.56*	12.62±1.35*

Supplementary Table 1. Functional and morphological analysis of aortic banded mice. Mice were subjected to aortic constriction for 1 week before intraperitoneal injection with vehicle. After 1 week (ie 2 weeks aortic constriction), echocardiography was performed. Mice were then killed and morphological analysis performed. Op, operation; HR, heart rate; IVS, interventricular septum thickness; LVPW, left ventricular diastolic posterior wall thickness; EDD, left ventricular end-diastolic dimension; ESD, left ventricular end-systolic dimension; FS, fractional shortening; EF, ejection fraction; AoPg, aortic pressure gradient; BW, body weight; HW, heart weight; LW, lung weight. *$p<0.05$ vs sham (ANOVA). # - AoPg was measured 1 week post-operation.

Suppl. Figure 1. Ng et al. 2010



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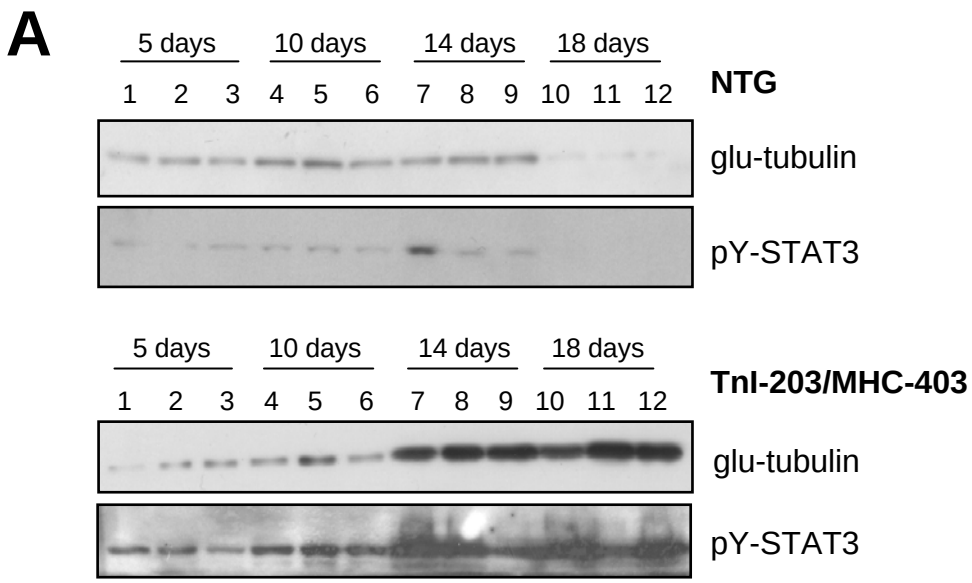

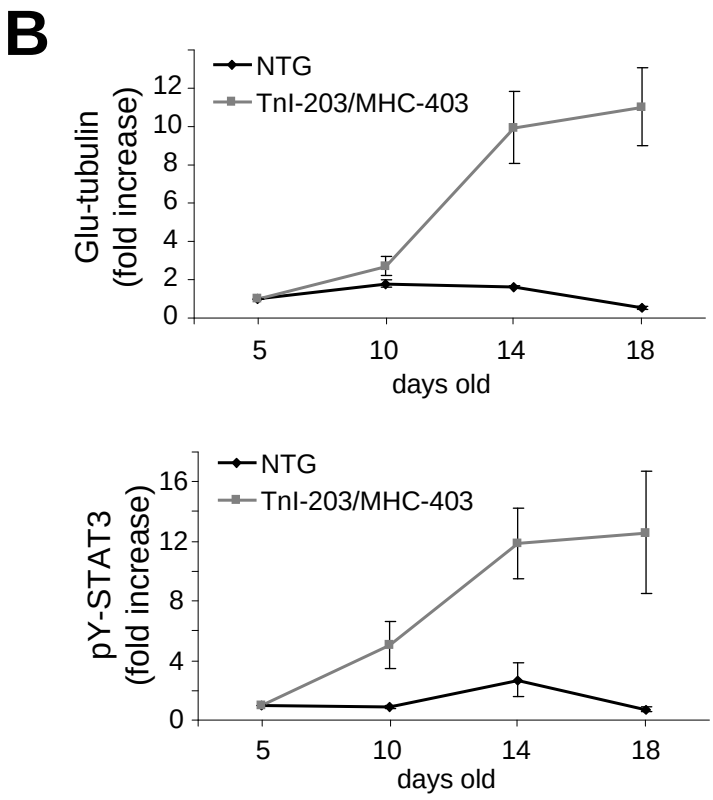

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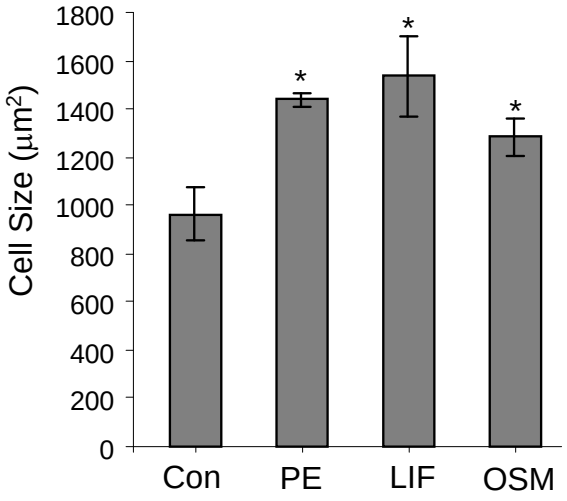

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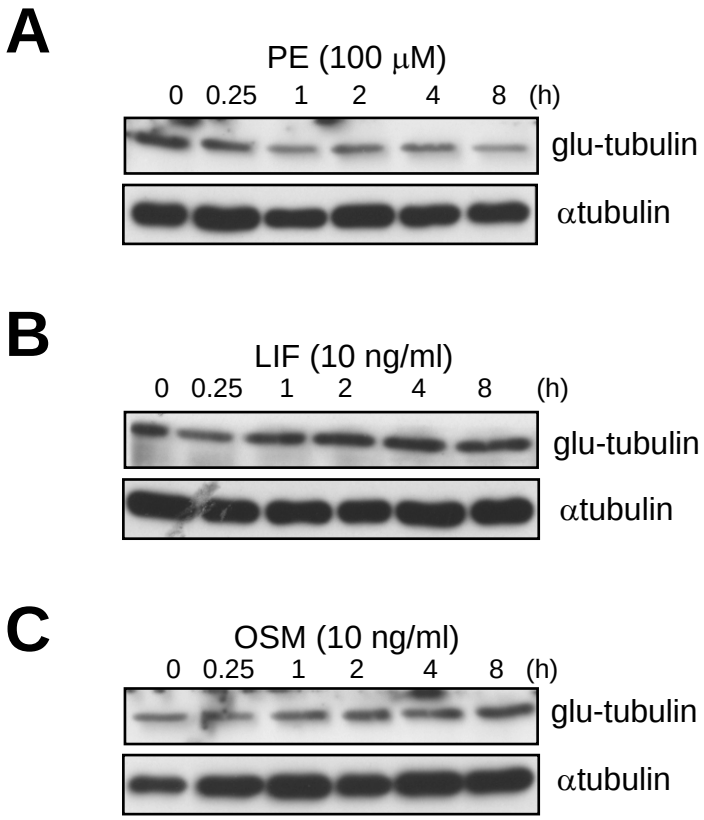

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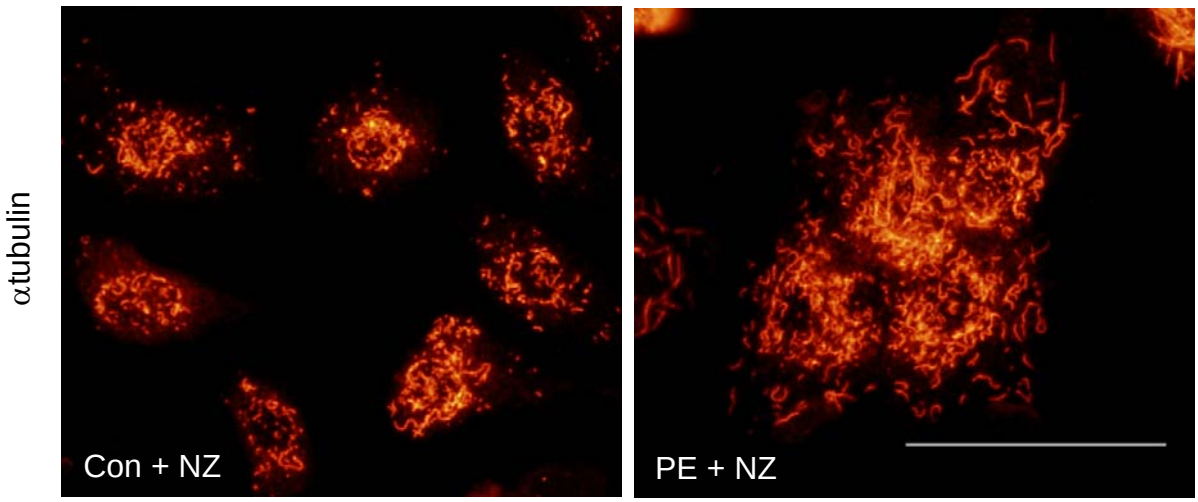

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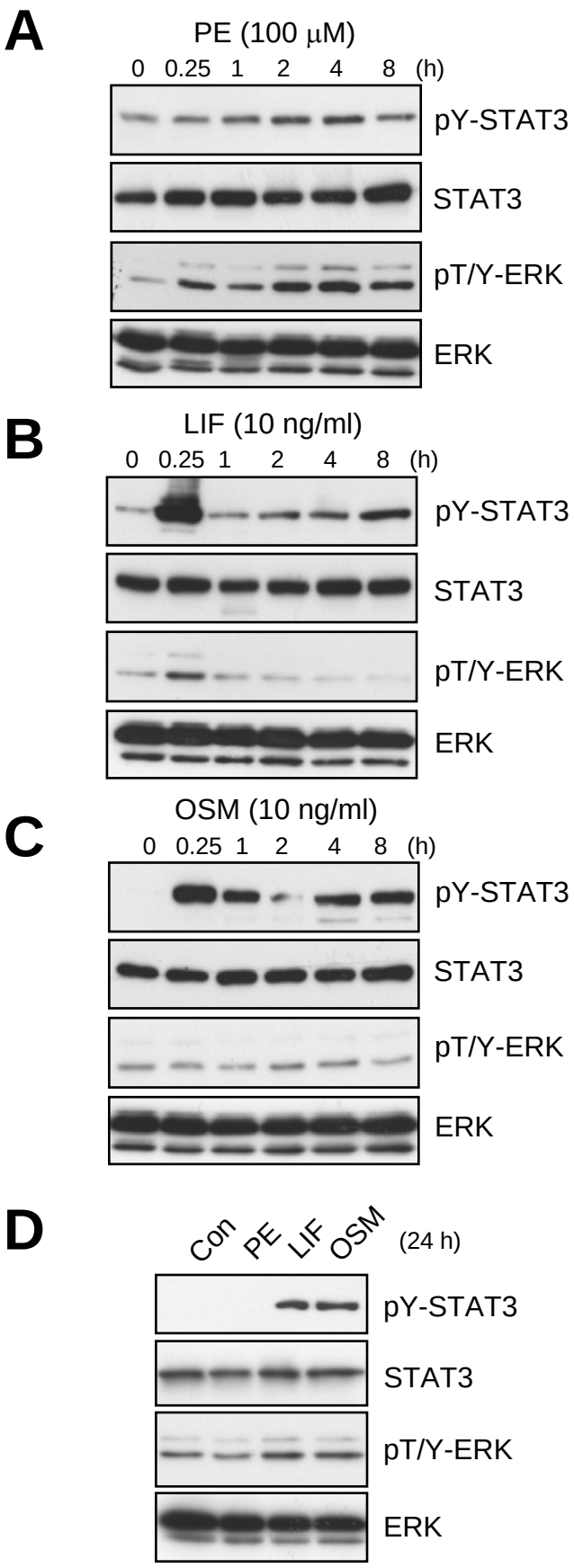

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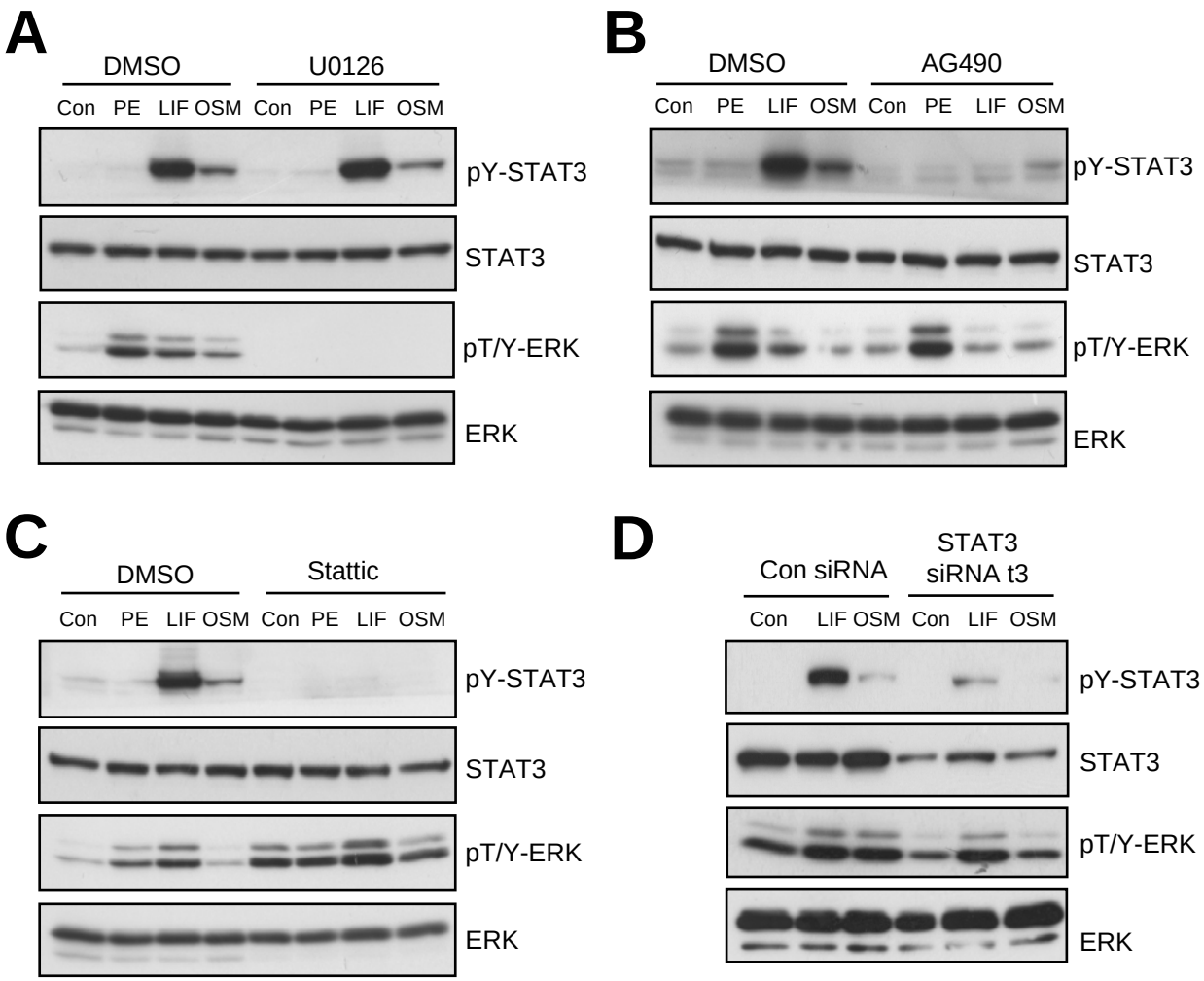

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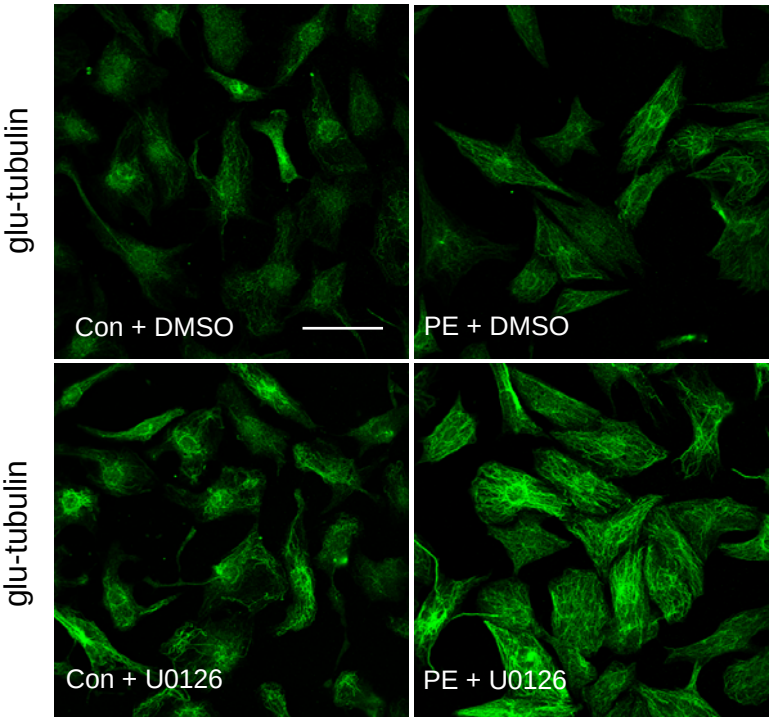

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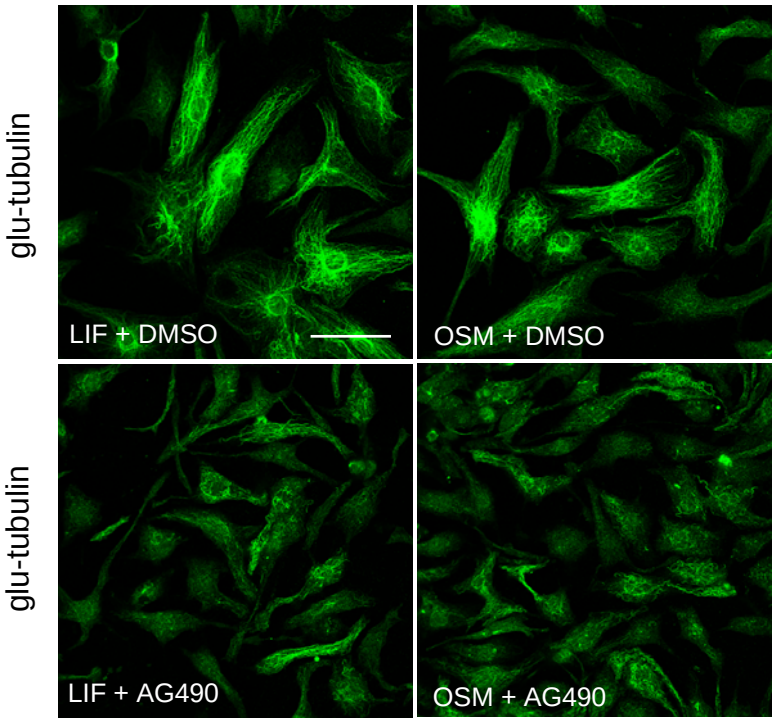

Suppl. Figure 10. Ng et al. 2010