Growth factor *erv1*-like Modulates Drp1 to Preserve Mitochondrial Dynamics and Pluripotency in Mouse Embryonic Stem Cells

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Supplementary Information:

Supplementary Figure 1. Depletion of Gfer results in significantly reduced marker gene expression in all three germ layers. Average (n=3), normalized (to actin) mRNA levels of Pax3 and FGF5 (markers of neuroectoderm), Gata4 and Sox17 (markers of endoderm), and Goosecoid and Brachury (markers of mesoderm) in day 6 EBs formed by indicated genotypes; Error bars represent ± SD; * p=0.00004.

Supplementary Figure 2. Lenti-GFP-shRNA-virus infection does not affect mitochondrial $\Delta\Psi_m$ of ESCs. Representative histogram (n=6) depicting TMRE fluorescence in lacZ-KD (red) and WT ESCs (green) at 72 hours post lentivirus infection.

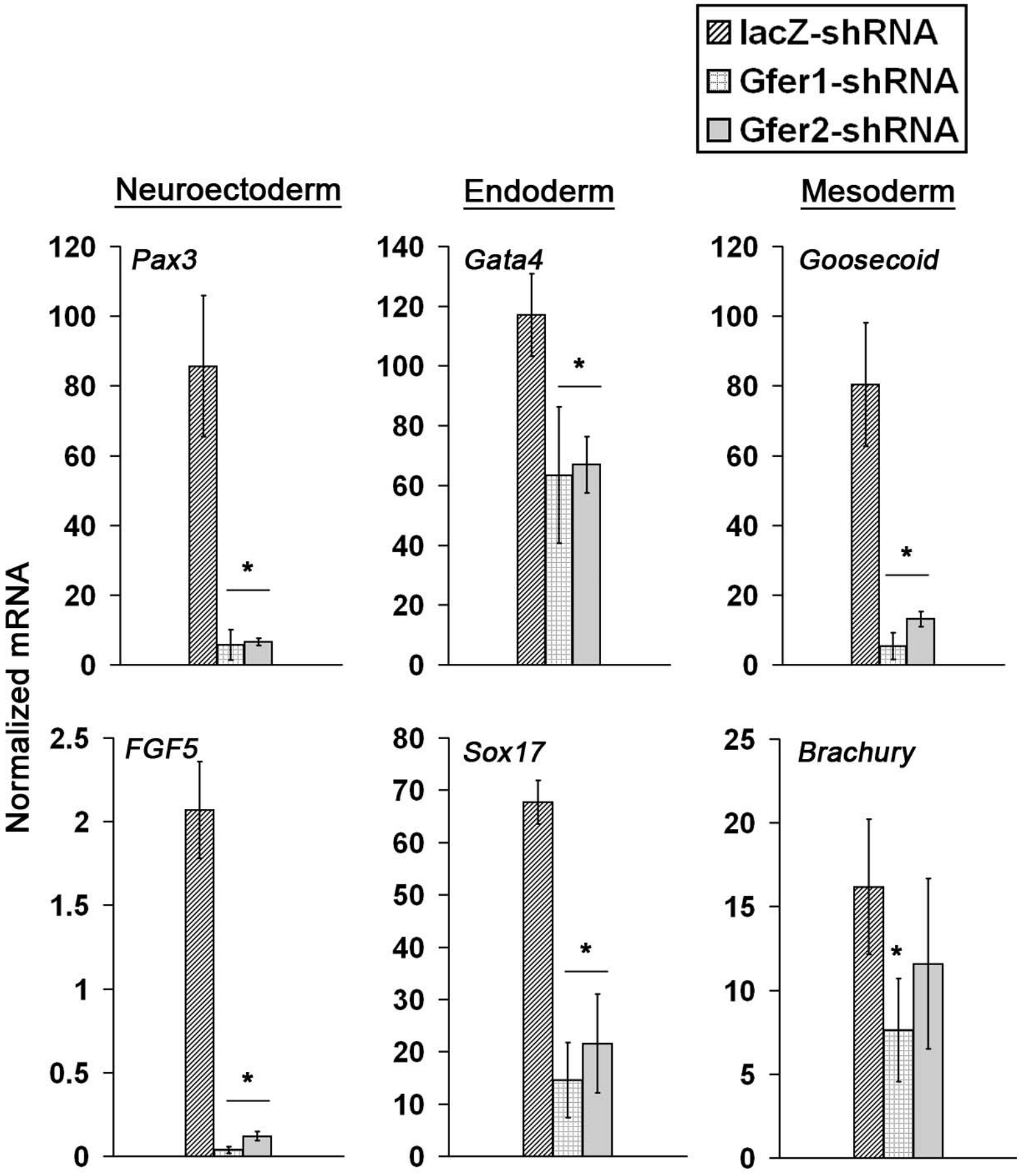
Supplementary Figure 3. Gfer KD initiates degeneration and autophagy of mitochondria in ESCs. (A) Digital TEM images depicting ultrastructural details in Gfer-1 KD ESCs. Scale bars are 5 μM at 2650X and 1μM at 7100X and 25000X magnifications. (B) Representative immunoblot (n=3) analyses of autophagy associated proteins Beclin-1, Atg5/12 and LC3b in indicated ESC genotypes. Gfer and actin levels are also shown.

Supplementary Figure 4. Inhibition of mitochondrial ETC complex I triggers mitophagy in WT-ESCs and Gfer overexpression protects ESCs from rotenone toxicity. (A) Representative histograms (n=3) showing % of apoptotic cells, measured by Annexin V/7-AAD reactivity in ESCs, at 24 h following treatment with DMSO (vehicle) or 10 μM rotenone. (B) Digital TEM images depicting ultrastructural details in ESCs at 24 h following treatment with DMSO or 10 μM rotenone. Images are presented as 2650X (scale 5 μm), 5600X and 25000X magnifications (scale 1 μm). (C) Percentage of apoptotic cells, measured by Annexin V/7-AAD reactivity in indicated ESCs genotypes, at 24 h following treatment with DMSO or 10 μM rotenone (n=3).

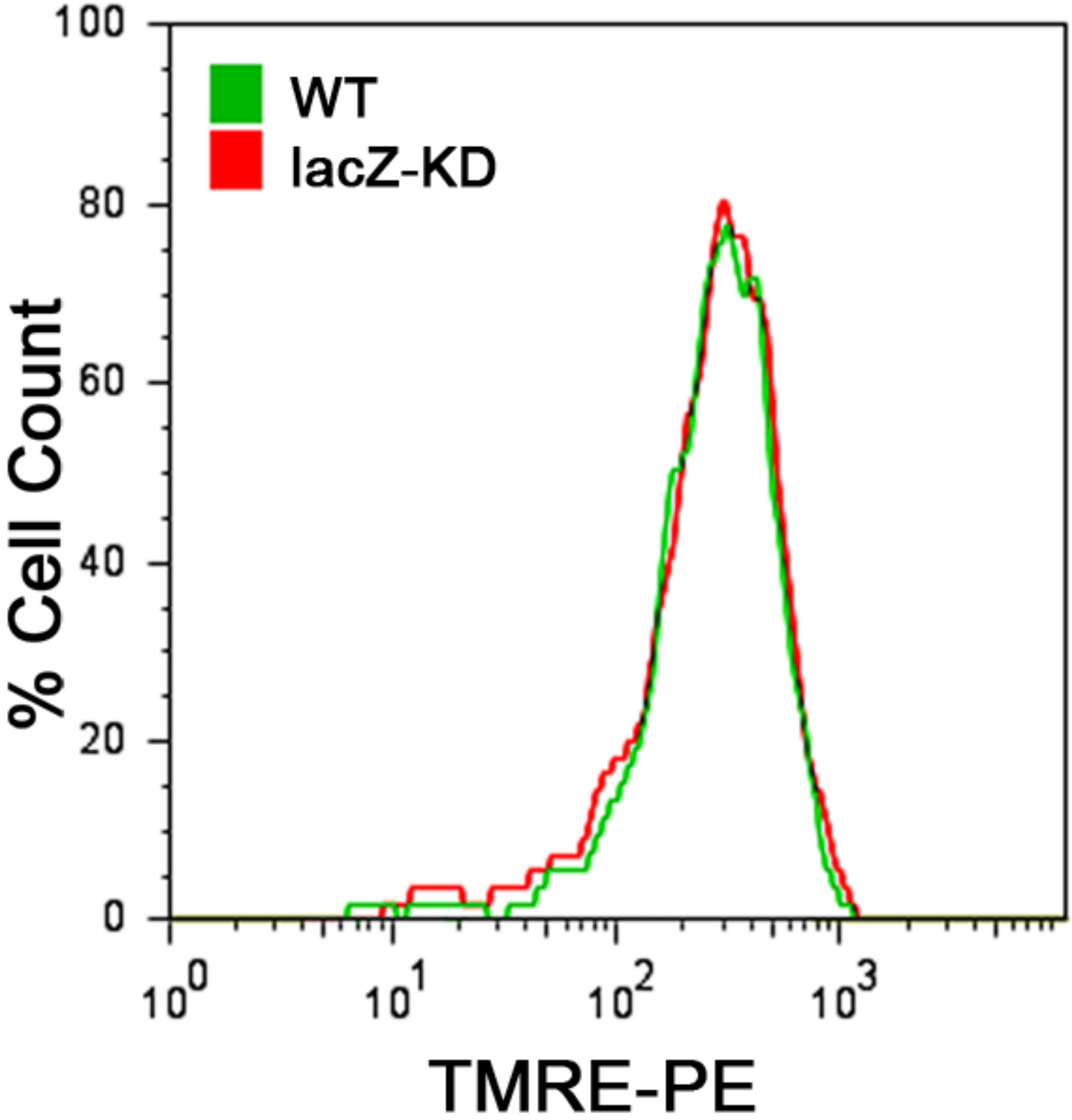
Supplementary Figure 5. Gfer does not modulate fusion GTPases and inhibition of Drp1 does not alter pluripotency marker expression in lacZ-KD ESCs. (A) Representative immunoblots (n=2) depicting OPA1, Mfn1, Mfn2 and actin protein levels in Control, Gfer-KD and MSCV-Gfer ESCs. (B) Digital ApoTome (optical section) images (630X magnification) depicting Nanog, Oct-4, and SSEA1 expression (all in Red; Dapi in blue) in lacZ-KD ESCs and lacZ-KD ESCs expressing $Drp1^{DN}$.

Supplementary Table 1: Sequence information of primers used in qRT-PCR.

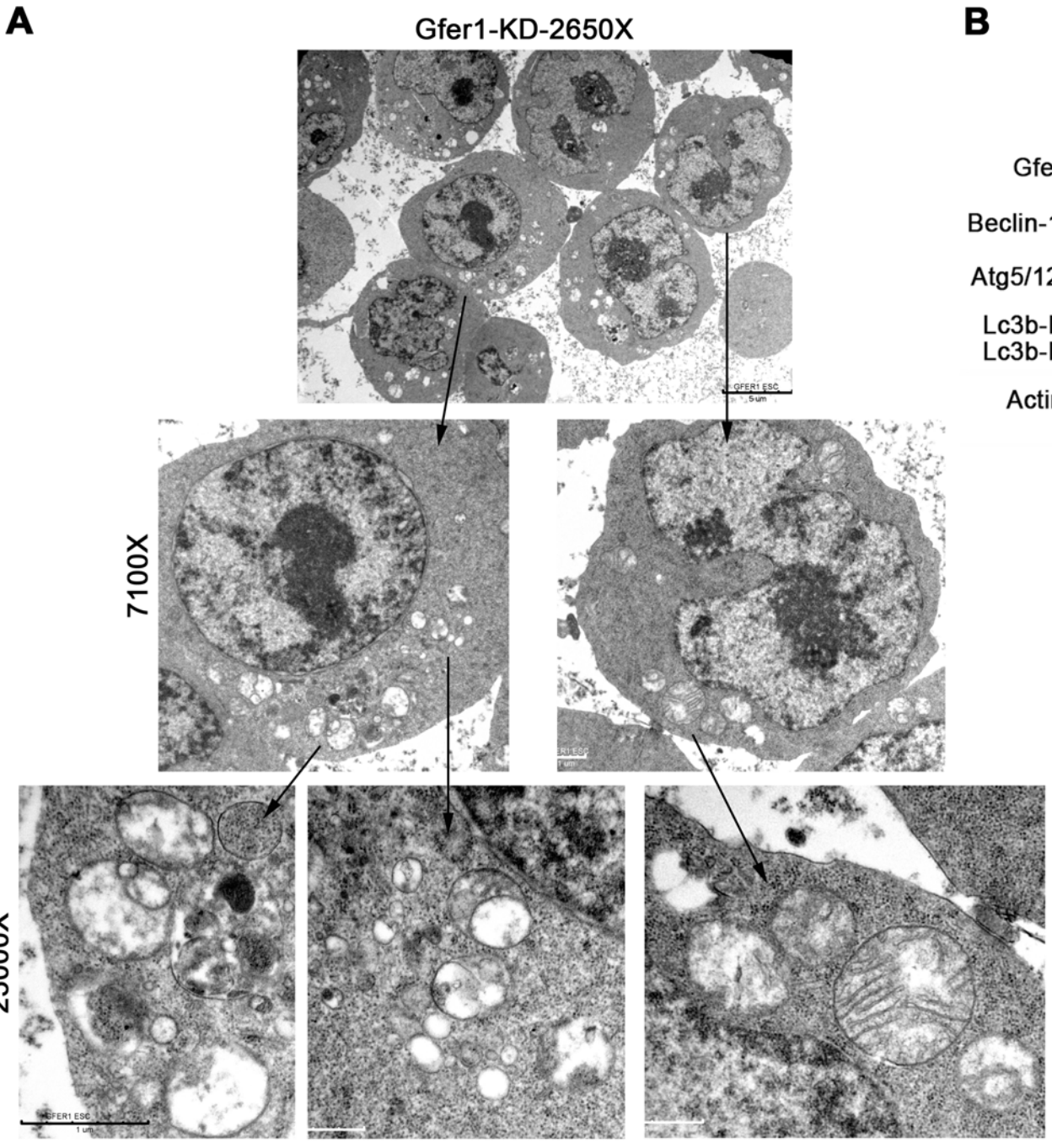
Supplementary Figure 1



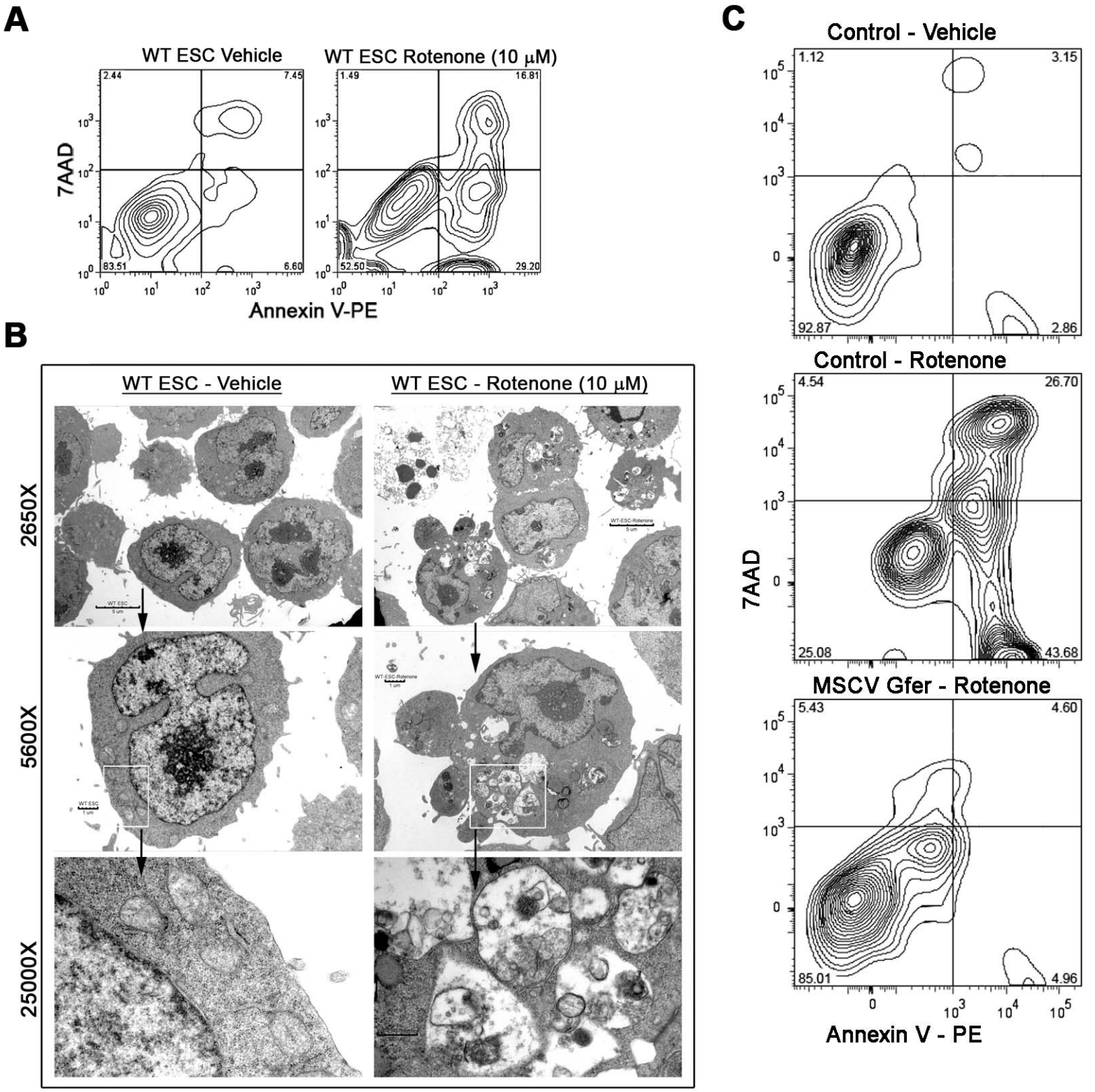
Supplementary Figure 2

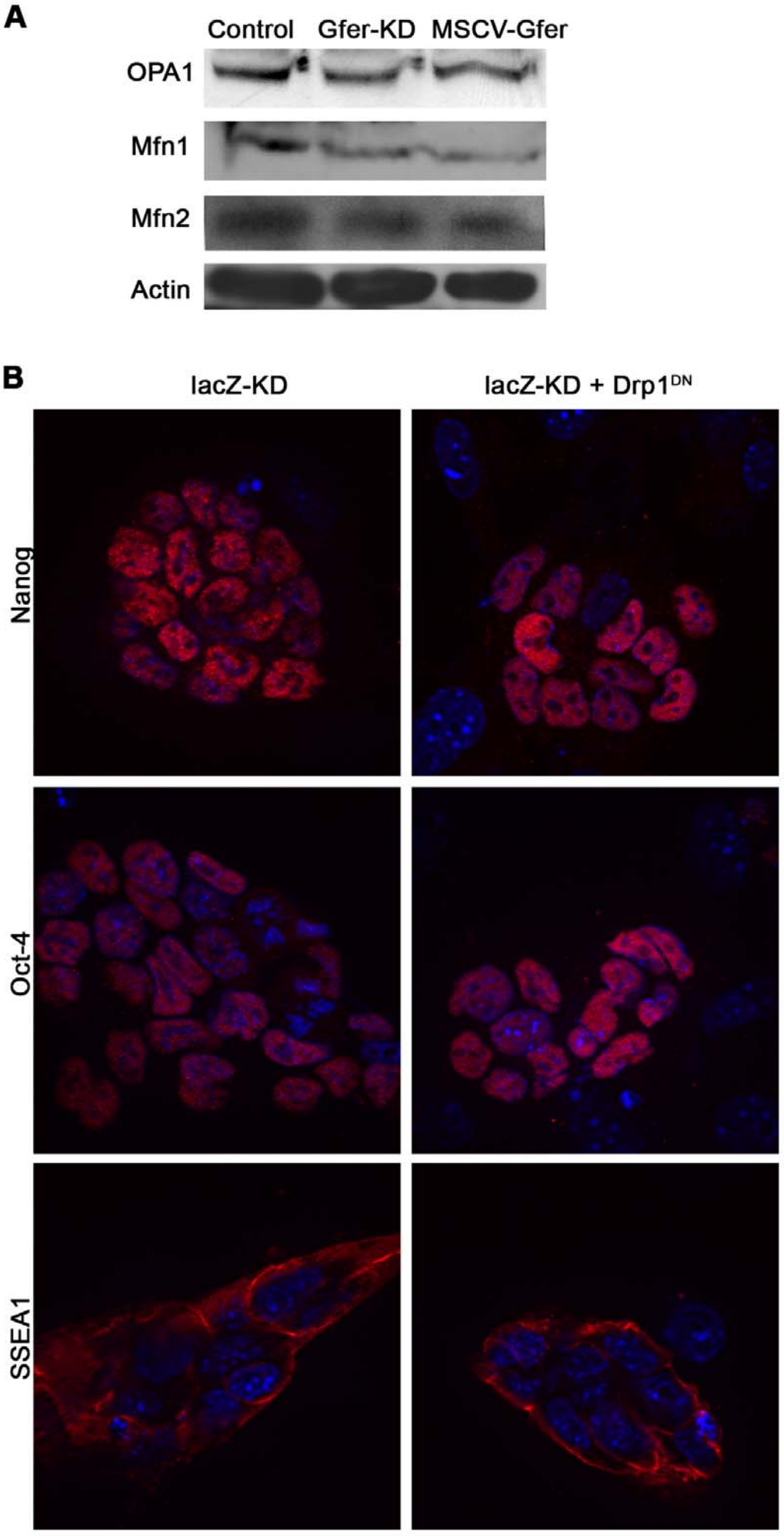


Supplementary Figure 3



Supplementary Figure 4




A
Control
Gfer-KD
MSCV-Gfer
OPA1
Mfn1
Mfn2
Actin
B
lacZ-KD
lacZ-KD + Drp1DN
Nanog
Oct-4
SSEA1

Supplementary Table 1: Quantitative RT-PCR Primer Sequences

Gene	qRT-PCR Primer (5'-3')
Actin-F	GGG AAATCG TGC GTG ACA TC
Actin-R	CCA AGA AGG AAG GCT GGA AAA G
Gfer-F1	TCTAGCCTGGTTCTATGGGCAACA
Gfer-R1	TCAGATGACAGCGCCTCTGAAACT
Brachury-F	CTC TAA TGT CCT CCC TTG TTG CC
Brachury-R	TGC AGA TTG TCT TTG GCT ACT TTG
FGF5-F	CTG TAT GGA CCC ACA GGG AGT AAC
FGF5-R	ATT AAG CTC CTG GGT CGC AAG
GATA-F	TTC CTG CTC GGA CTT GGG AC
GATA-R	TTC CCA GGC AGG TGG AGA ATA AG
Pax3-F	ATA AGC CCA GGA CAC AGA GTT GTG
Pax3-R	GTC TAG TCT GTG GAG GCC GGA AAC
Sox17-F	AAG AAA CCC TAA ACA CAA ACA GCG
Sox17-R	TTT GTG GGA AGT GGG ATC AAG AC
Goosecoid-F	AAA CGC CGA GAA GTG GAA CAA G
Goosecoid-R	AAG GCA GGG TGT GTG CAA GTA