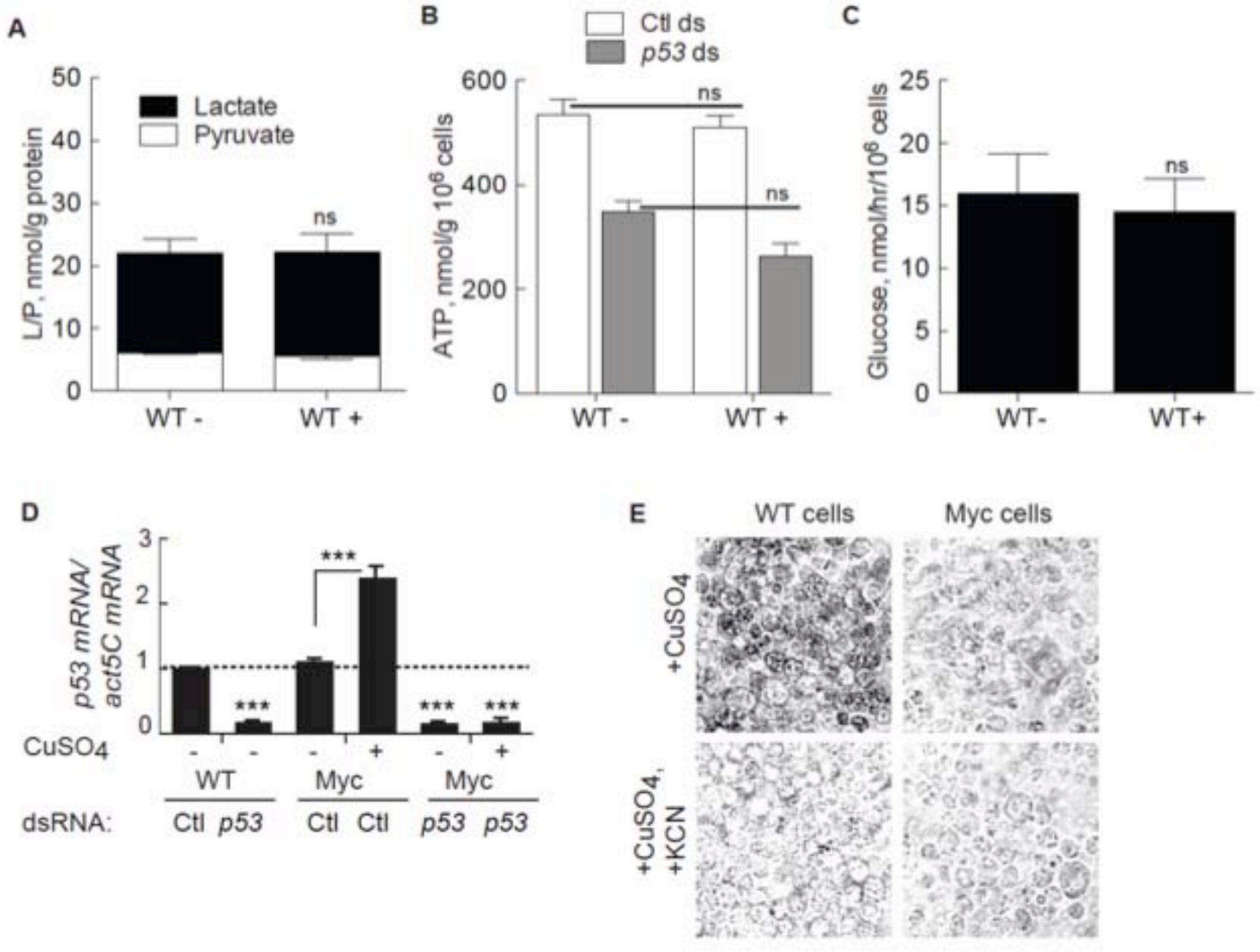
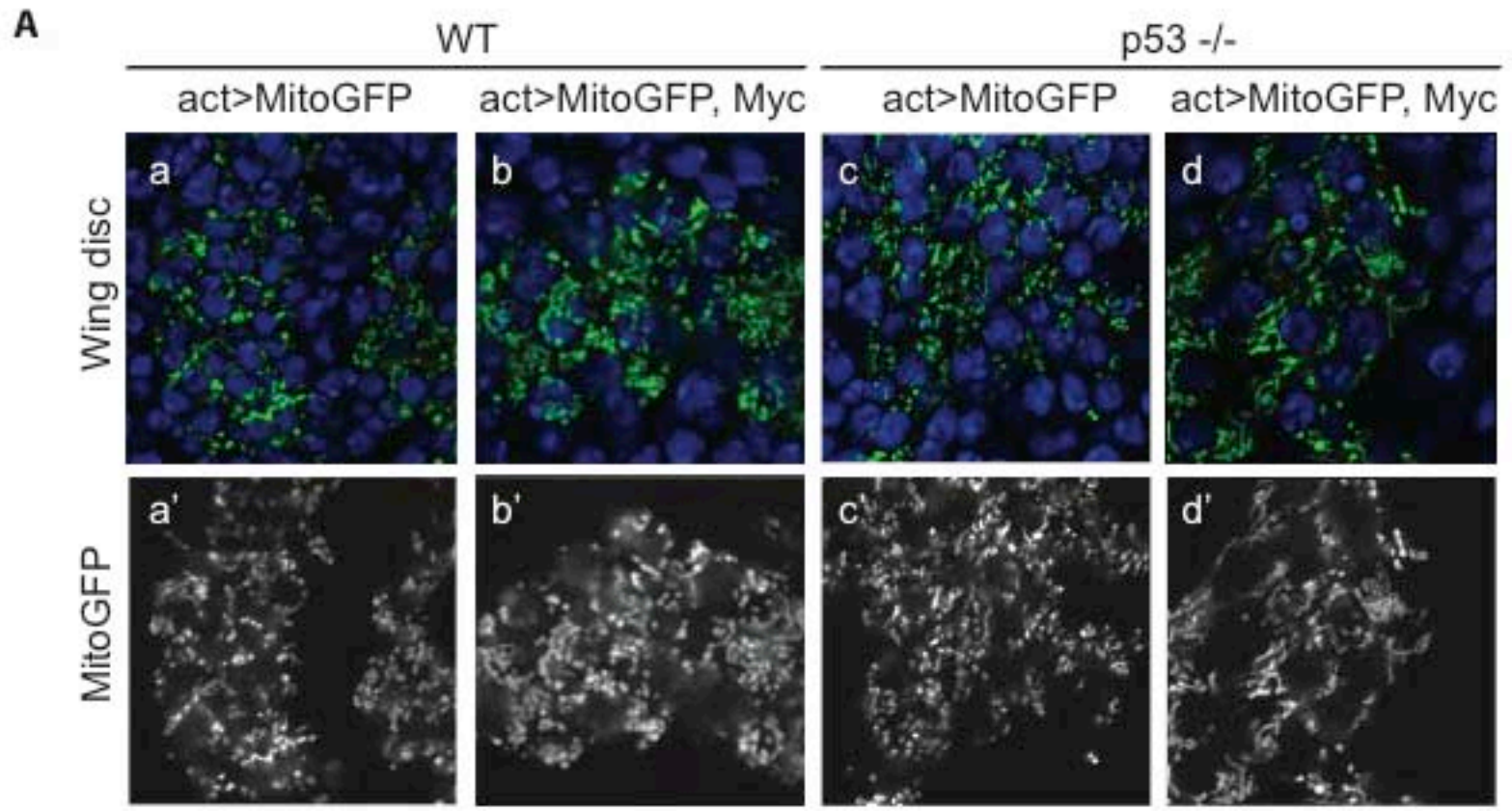
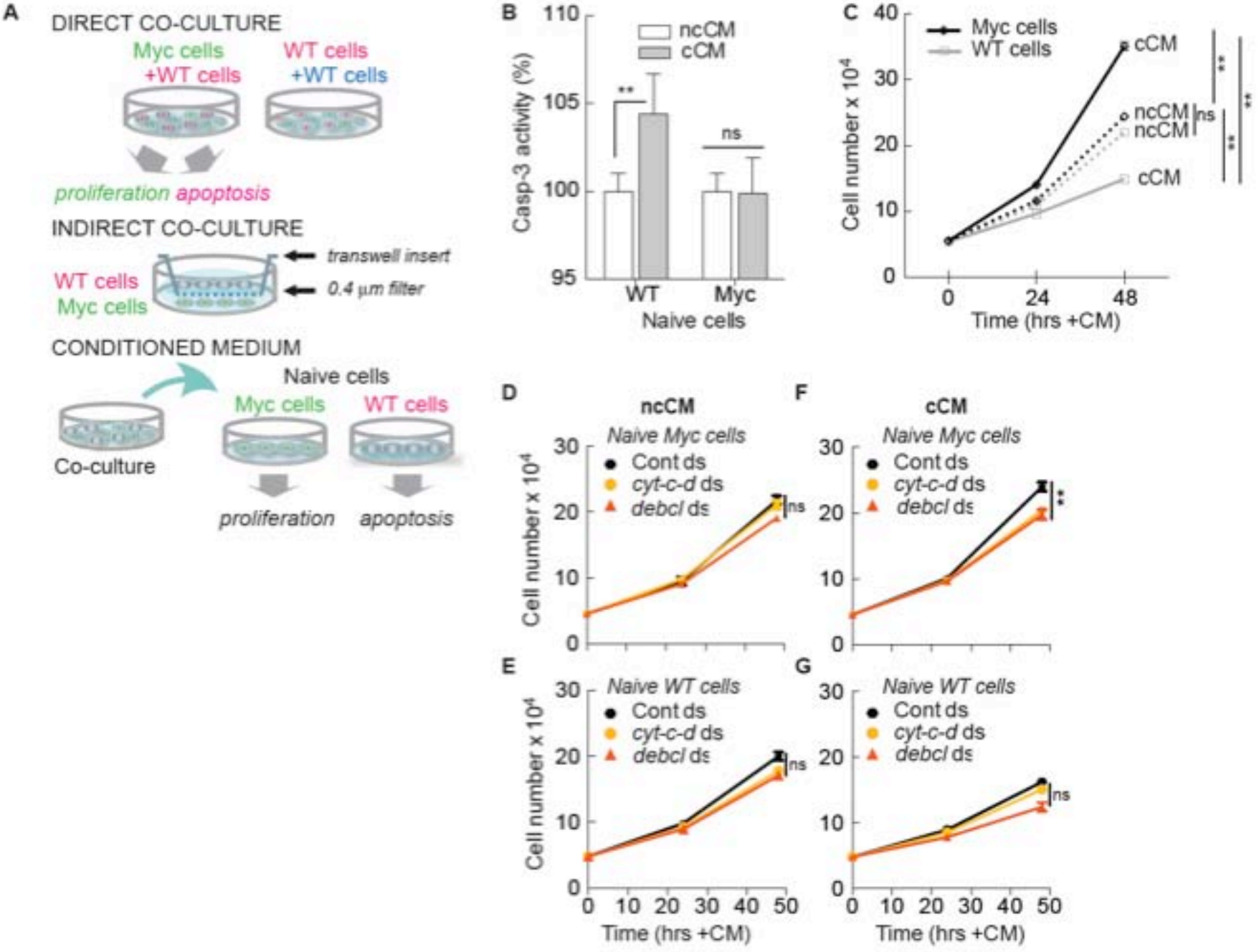
Supp Figure 1



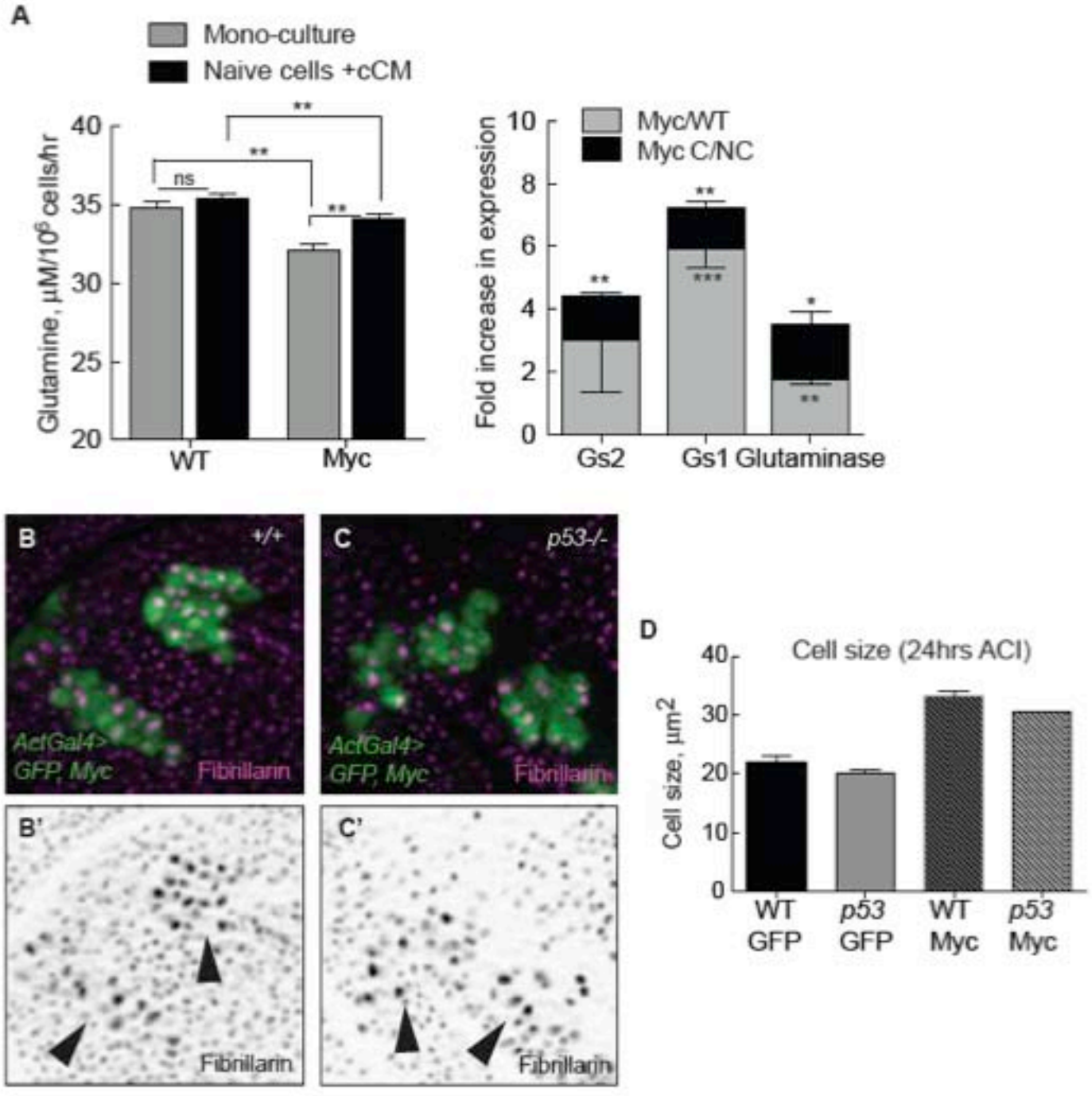
Supp Figure 2.



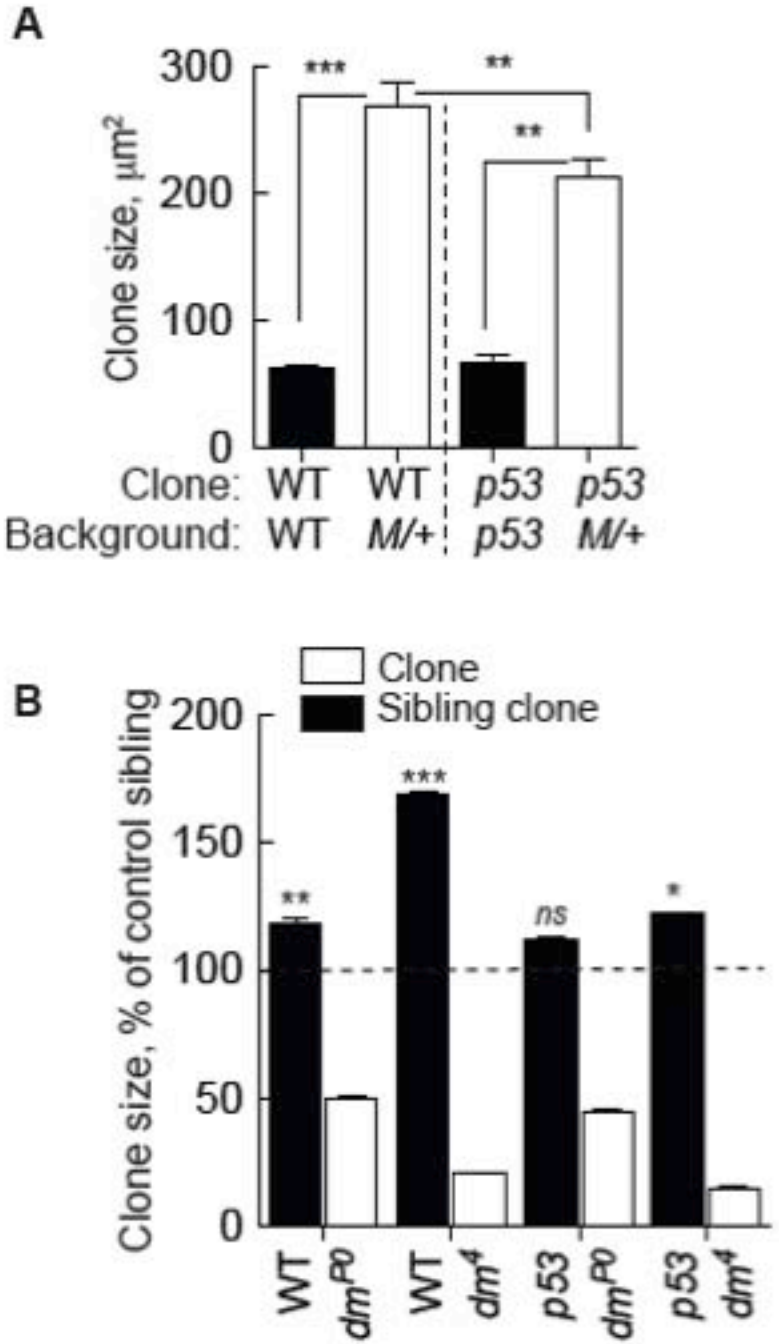
Supp Figure 3.



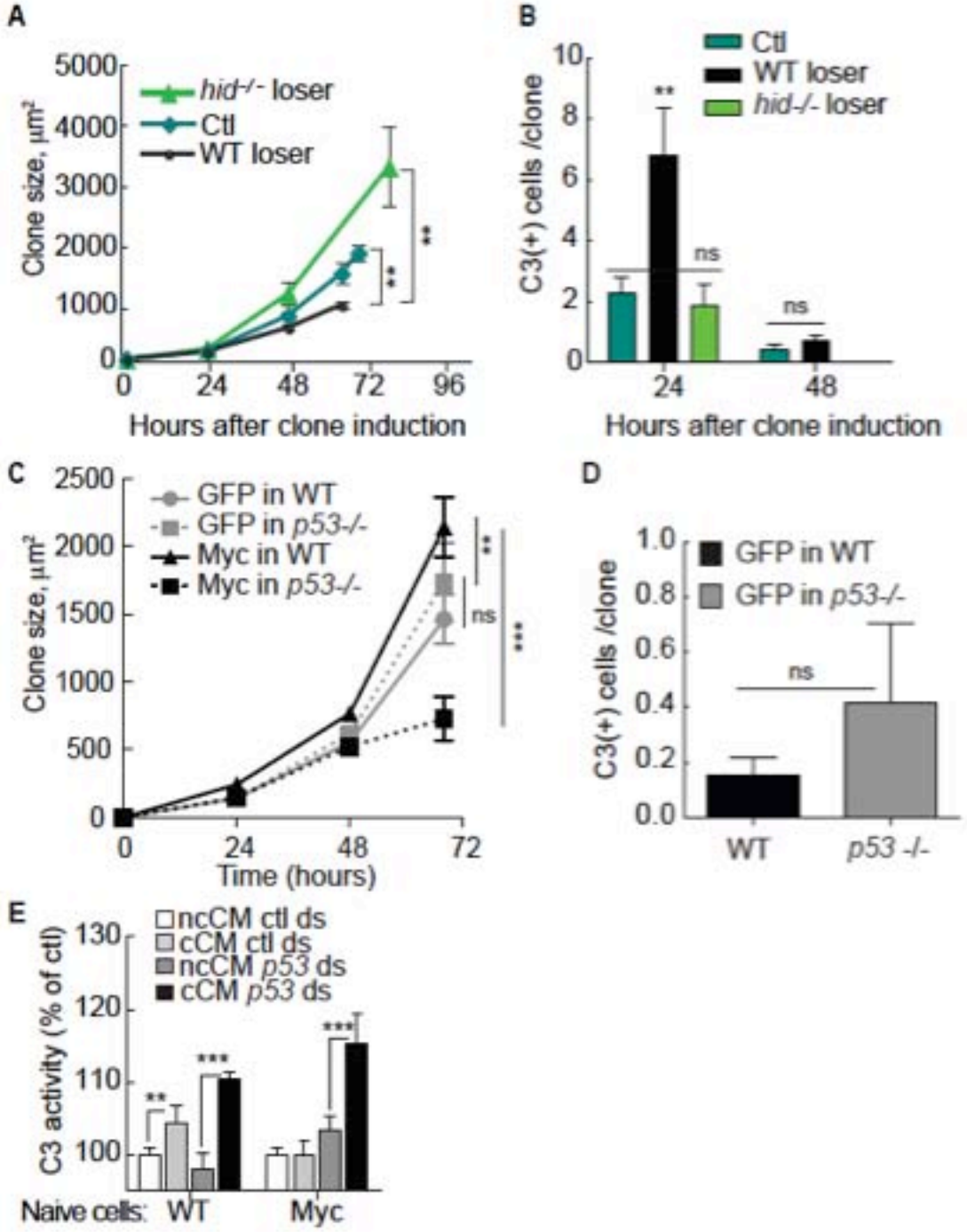
Supp Figure 4



Supp Figure 5



Supp Figure 6



Supplementary Materials.

Supplementary Figure Legends:

Figure S1, related to Figure 1. S2 cell metabolism is not significantly altered by CuSO4 treatment.

A. Intracellular lactate and pyruvate in WT S2 cells grown with (+) or without (-) 125μM $CuSO_4$. Error bars, SD.

B. Steady-state ATP in WT cells treated with control dsRNA or p53 dsRNA. Although p53-deficiency reduces ATP, the effect of 125μM $CuSO_4$ is negligible. Error bars, SD.

C. Glucose consumption in WT cells is not affected by addition of 125μM $CuSO_4$. Error bars, SD.

D. q-RT-PCR showing that *p53* dsRNA efficiently knocks down p53 expression in WT cells and Myc cells. CuSO4 was used to induce Myc expression in Myc cells and was also included in WT cell cultures as a control. Error bars, SD.

E. Histochemical COX assay on WT and Myc S2 cells. Both cell types were treated with 125μM $CuSO_4$. Cox activity is reduced in Myc cells. Bottom panels: as a control, cells were treated with KCN to eliminate COX activity.

Figure S2, related to Figures 1 and 2. Mito-GFP in wing disc clones.

A. Mito-GFP labeled mitochondria in wing disc clones. a) control WT clones. b) Myc-expressing clones. c) control clones in *p53* mutant wing disc. d) Myc-expressing clones in *p53* mutant wing disc. Single Mito-GFP channels are shown below. Wing disc cells are small and reside within a pseudo-stratified columnar epithelium, making mitochondria more difficult to visualize than in fat body cells (**Figures 1H & 2F**).

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Figure S3, related to Experimental Procedures and Figure 4. S2 cell competition assays and the effect of competitive versus non-competitive CM.

A. S2 cell culture cell competition assays. For more details see Methods, and Senoo-Matsuda and Johnston, 2007. In direct co-culture, Myc and WT cells are cultured together, in the presence and absence of $CuSO_4$ inducer. Competitive co-cultures are those with $CuSO_4$. Control co-cultures of only WT cells are treated identically. In indirect co-culture, the different cell populations are separated by a 0.4um filter but grown in the same culture dish. Physical separation of the two cell populations allows analysis of each population individually and was used in some metabolic assays. In the CM assay,

direct co-cultures are used to generate CM from either competitive or non-competitive cultures. The CM is added to naïve cells and the growth or survival response of each population is assessed.

B. Naïve WT cells, but not naïve Myc cells, increase C3 activity in response to cCM. ncCM does not induce C3 activity in either cell population. C3 activity was assessed by enzymatic assay. Error bars, SD.

C. Myc cells increase their rate of proliferation in response to cCM but not ncCM, while the proliferation rate of WT cells is decreased. The reduced proliferation of WT cells is due to increased cell death and is completely suppressed by dsRNA against Hid (Senoo-Matsuda and Johnston, 2007). Error bars, SD.

D-G. CM made from competitive and non-competitive co-cultures treated with dsRNA against *cyt-c-d* or *debcl.* Naïve assay cells were also treated with dsRNA. Knockdown of either gene does not affect proliferation Myc **(D)** or WT **(E)** cells in the absence of competition (ncCM), nor did cCM affect proliferation of WT cells **(G).** However, both dsRNAs prevent the accelerated proliferation of naïve Myc cell**s (F).** Error bars, SD.

Figure S4, related to Figure 3. Glutamine consumption is not limiting for growth of Myc cells and ribosomal-regulated cellular growth is not impaired in *p53* mutant Myc- expressing wing disc cells.

A. Left, glutamine consumption in WT or Myc cells in mono-culture, and in naïve WT or Myc cells treated with cCM. Glutamine consumption is reduced in Myc cells relative to WT cells under both conditions. Glutamine consumption is higher in Myc super-competitors than in Myc cells from mono-cultures, but not higher than WT cells. Right, results of qRT-PCR experiments showing that glutamine synthase 1 (Gs1), glutamine synthase 2 (Gs2) and glutaminase mRNAs are increased in Myc cells relative to WT cells (grey bars) and their expression is enhanced in Myc cells during competition (black bars). Error bars, SD.

B-C. Loss of *p53* does not prevent regulation of cellular growth by Myc in wing discs. **B-B'**. Fibrillarin expression (magenta in **B**, black in an inverted image in **B'**) in Myc-expressing wing disc clones (green). The nucleolar size is increased and the nucleolar protein Fibrillarin (arrows) is induced by Myc in both WT (+/+) and *p53* mutant backgrounds (**C-C'**).

D. Cell size is increased by Myc activity regardless of *p53* status. Cells were measured 24 hr after clone induction (ACI) in wing discs. Error bars, SEM.

Figure S5, related to Figure 4. Full winner status requires p53.

A. Cell competition between *M(3)RpS3/+* cells and either clones of WT cells or clones of *p53* null mutant cells. Graphs show the mean size of WT clones in a WT background, WT clones in a *M(3)RpS3/+* background, *p53* mutant clones in *p53* mutant background, or *p53* mutant clones in a *M(3)RpS3/+* background. WT clones and *p53* mutant clones grow equally well in a homotypic background (black bars). WT cells are strong competitors against *M(3)RpS3/+* cells, and occupy large territories in the discs at the expense of the *M(3)RpS3/+* cells (white bar, WT in *M/+*). By contrast, *p53* mutant "winner" clones in the *M(3)RpS3* heterozygous background (white bar, *p53* in *M/+*) are significantly compromised in their growth during the same period of time. All clones were induced in parallel and grew for 40 hours. Error bars, SEM.

B. Competition between WT and *dm* mutant cells. Sibling clones of WT cells (black bars) "win" and proliferate at an accelerated rate when growing next to *dm* mutant cell clones (white bars). Data is presented as percent of the appropriate control WT clone size, and genotypes of paired clones and sibling clones are noted. dm^{P0} is a hypomorphic allele; dm^{4} is a null allele. *dm* clones were generated in a homozygous *p53* null mutant background. Loss of p53 prevents the sibling "winner" cells from proliferating faster than control sibling clones. All clones were induced by mitotic recombination in parallel and grew for the same period of time. Error bars, SEM. Significance is relative to control sibling clones (dotted line marks100%).

Figure S6, related to Figure 5. Loser cell death requires the HID pro-apoptotic factor and p53-mediated survival of Myc cells depends upon the cellular environment.

A. Growth curves of wing disc cell clones. Control GFP cells (black) and GFP loser cells (dark green) in a WT background compared to GFP loser cells in a *hid* mutant background (light green). Error bars, SEM.

B. In WT wing discs loser GFP clones contain numerous C3 positive cells compared to control GFP clones. In *hid* mutant discs the number of C3 positive cells within GFP loser clones is significantly reduced at 24 hours after clone induction and completely absent by 48 hours. Error bars, SEM.

C. *p53* mutant *tub>myc>Gal4* cells do not undergo apoptosis in the absence of cell competition. A control experiment in which *p53* mutant, *tub>myc>Gal4* expressing wing

discs were heat-shocked but lacked HS-Flp recombinase, thus no loser GFP clones were generated. Very few C3-positive cells (red) are observed.

D. Growth of competitive Myc-expressing clones, but not control, GFP-expressing clones, is reduced in wing discs from *p53* mutant larvae. Error bars, SEM.

E. C3 activity (ELISA assay, as a percent of control) in *p53*-depleted, naïve WT or Myc cells treated with cCM or ncCM from control or *p53*-depleted co-cultures. C3 activity is also increased in the naïve WT cells by *p53* depletion. Error bars, SD.

Supplementary Table 1, related to Experimental Procedures.

Supplementary Table 1. Primer sequences used in qRT-PCR experiments

Gene name	CG number	Forward primer	Reverse primer
Glut1	CG1086	TGATGCAGCTGAGCCAGCAA	TCCAGGGCAGTCTTCATGCT
Glut3	CG3853	TCTTCGAGGAAGAGTGGCAC	TTCGGCAGCAACTGGATGAG
HK-A	CG3001	GGTGCACGAGTTATGTCAGC	GTGCGATGGCATCCTTTAGC
HK-C	CG8094	GAGGTGCGAGAACTTATGCA	GAATCCCTTAGTCCAGCGTA
Pgi	CG8251	GGCAAACCCGTCAAGTACAG	GCCATTAAAGCCTCCGTCTG
Pfk	CG4001	CATCACTGTCTTGGGTCACG	ATCACAGCTAGGCGGTATCC
Ald	CG6058	CATTCTGGGCATCAAGGTCG	GGATCGACTGGTAGGATGGG
Tpi	CG2171	ATCAGGCTCAAGAGGTCCAC	GCGTTGATGATGTCCACGAA
Eno	CG17654	CCCGTCAGATCTACGACTCC	GATTGGCCTTGATCAGCTCG
Ldh/Impl3	CG10160	TGGTAGAGTACAGTCCCGAT	ACCACGGATGTCACACCGTT
Pdh	CG7010	ATCAGGGACAGGTGTTCGAG	CCAAAGGTCCGTGAGTGTTC
Sdh	CG3283	TCGAGATCTACCGCTGGAAC	AGCGACTTGGAGGTGTTGAT
Scox	CG8885	CAGGGAGCTGTTCGCAAATC	CATGATGATGGTGTGATCCAC
GS1	CG2718	TGCGTCTGCTGCGTACTGGC	CGGCGTTTCCAGGTTGCGGTA
GS2	CG1743	GGACACCTACCTGTATCCGG	ATGGCCATCAAAGTCGAGGA
Glutaminase	CG42708	ATCGTCGTCATTAGGGCAAC	CGCTTCAAAGATCCGTCTC
p53	CG33336	ACTCCGGGAATCTGATGCAG	TCAAAGGCTCAACGCTAAGG
Myc	CG10798	CGCTCTGATCCGTATTCCAT	CGCTTCTGACAGACCGTGTA

Supplementary Experimental Procedures:

Fly strains and husbandry. Strains used in this study include, *yw*hsflp^{122}; act>*y*,*stop*>Gal4, UAS-GFP, *yw*;*tub>myc, y>Gal4* (de la Cova et al., 2004), *yw*hsflp^{122};UAS-GFP, *yw*hsflp^{122};UAS-RFP, *rpr-150-lacZ;*+/SM6-TM6B (Brodsky et al., 2000), *p53*^{ns} (Sogame et al., 2003), *p53*$^{5A\text{-}1\text{-}4}$ (Rong et al., 2002), *yw*hsflp^{122};UAS-Myc (Johnston et al., 1999), *yw*hsflp^{122} tub-Gal4,UAS-GFP;;FRT82B tub-Gal80 hs-CD2 (de la Cova et al., 2004), Mito-GFP, *RpS3*^{Plac92} and *Ubi-GFP* FRT82B *arm-lacZ*, (from the Bloomington Stock Center). *yw Ubi-GFP* FRT19A*;hsfl*p^{1} (gift of K. Basler), *w dm*^{P0} FRT19A*/FM7c*, *w dm*^{4} FRT19A*/FM7* (gift of P. Gallant). Eggs from appropriate crosses

were collected on freshly yeasted grape plates for short periods (2-4 hours). After hatching, larvae were transferred to standard molasses food vials (≤ 50/vial) supplemented with fresh yeast and raised at 25ºC for defined periods of time.

Additional information for generation of somatic clones. For the *RpS3/+* experiment, WT and *p53*^{ns} mutant clones were generated in *RpS3/+* larvae by HS at 37°C for 10 minutes at 84 hours AEL, and in control WT larvae by HS at 72 hours AEL (the timing difference was based on the developmental delay of *RpS3/+* larvae, determined experimentally by measuring wandering and pupariation times of each genotype). Clones in each genetic background were allowed to grow for a 40 hour period at 25°C. For the *dm* experiment, *dm*^{P0} FRT19A (hypomorphic) or *dm*^{4} FRT19A (null) mutant, or WT FRT19A control (zero-copies GFP) and sibling FRT19A Ubi-GFP (marked by two-copies GFP) clones were induced by Flp/FRT-mediated mitotic recombination in *dm*/+ or *dm*/*p53* (one copy GFP) wing discs after larval HS in at 37°C for 40 minutes at 48h AEL, and allowed to grow at 25°C until 112h AEL.

Antibodies used. After fixation of S2 cells, imaginal discs or fat body cells, Hoechst 33258 was used as a DNA counterstain. The following antibodies and dilutions were used: Mouse anti-Digoxigenin, 1:2000 (Roche); Rabbit anti-Cleaved Caspase-3 (C3), 1:100 (Cell Signaling), Mouse-anti rat CD2, 1:400 (Serotec), Rabbit-anti ß-gal, 1:2000 (Cappel), Mouse-anti ß-gal, 1:1000 (Promega). Secondary antibodies used were purchased from Jackson Immunoresearch and Molecular Probes.

Details of dsRNA treatment. Cells were plated for mono-, indirect, or direct co-culture (See Supp. Figure 3), and cultured with dsRNA with or without 125 µM $CuSO_4$ for 24 hours, and total RNA, cellular lysate, and CM was collected for various analyses. ncCM was made from control co-cultures (WT + WT cells with CuSO4 induction, or WT cells + Myc cells without CuSO4 induction; they behaved very similarly. However, to control for any non-specific effects CuSO4, we generally used uninduced WT + Myc co-cultures as our controls. In all experiments using CM, naïve cells were also pre-treated with the appropriate dsRNA. Growth assays were performed with cell counts in cultures at 24-hour intervals after adding CM.

Mitochondrial respiratory chain complex activity assays. Briefly, cells treated as indicated were suspended in 500 µl of ice-cold PBS and sonicated. Complex I and III (I+III) activity was measured by observation of the reduction of cytochrome *c* (cyt *c*) at 550 nm. Samples were incubated at 30° C in a medium containing 100 mM KH_2PO_4 (pH 7.5), 10 mM KCN, 2mM NADH, and 1mM cyt *c*. The increase of absorbance was observed for 1min, and 2 additional min after addition of rotenone (10 µg/ml). Complex I+III activity was calculated by subtracting activity in the presence of rotenone from total activity and expressed as µmol reduced cyt *c*/min/mg protein. Complex II and III (II+III) activity was measured by observation of the reduction of cyt *c* at 550 nm. Samples were incubated at 30° C in medium containing 100 mM KH_2PO_4 (pH7.5), 30 mM succinate, 10 mM KCN, and 1mM cyt *c*, and absorbance monitored for 2 min. The results were expressed as µmol reduced cyt *c*/min/mg protein. Cyt *c* oxidase (COX or complex IV) activity was measured following the oxidation of cyt *c* catalyzed by cytochrome oxidase. Samples were incubated at 30 °C in medium containing 10 mM KH_2PO_4 (pH7.0) and 1% reduced cyt *c*, and the reaction followed for 3 min at 550nm. The results were expressed in µmol oxidized cyt *c*/min/mg protein. Citrate synthase (CS) activity was measured following the reduction of 1 mM 5,5'-dithiobis(2-nitrobenzoic acid) in 1MTris–HCl (pH 8.1) at 412 nm (30 °C) for 2 min in the presence of sample, 10 mM acetyl-CoA, and 10

mM oxalacetic acid. CS was expressed as µmol/min/mg protein. Succinate dehydrogenase (SDH) activity was measured following reduction of 2,6 dichlorophenol-indophenol (DCIP) at 600nm for 1 min. Samples were incubated at 30 °C in medium containing 0.1M KH_2PO_4 (pH7.0), 400mM succinate, 0.015% 2,6 dichlorophenol-indophenol, and 10mM KCN. SDH was expressed as µmol/min/mg protein. The results of complexes I+III, II+III, and IV were normalized to CS activity.

Histochemical measurements of Sdh and COX activity were performed on S2 cells as follows: WT or pMT-Myc S2 cells were plated on coverslips at a density of $8x10^5$ in Schneider's medium supplemented with 10% FBS; after 16 hours Myc expression was induced with 125µM $CuSO_4$. 24 hours later cells were washed in PBS and air dried at RT for 1 hour. To measure COX activity, the cells were pre-incubated with 0.28 mg/ml cobalt chloride/50mM Tris-HCl (pH 7.6)/ 10%sucrose (Solution 1). Cells were equilibrated with 50ul DMSO/10ml medium for 15 minutes, then rinsed with 0.1M sodium phosphate (pH7.6)/10% sucrose for 5 minutes (Solution 2), and then incubated in pre-filtered medium at pH 7.5 containing 0.1M sodium phosphate/10% sucrose, cytochrome c type IV (Sigma C-7752), DAB hydrochloride (Sigma D-5905), and catalase (Sigma C-40), and DMSO (Solution 3) for 1-2 hours at room temperature; washed with Solution 2, then PBS, and finally water, and mounted in gelatin for microscopic examination. As a control, 10mM KCN was added to Solution 2 to kill COX activity in parallel reactions. SDH staining was carried out using Solutions 1 and 2 above to pre-incubate the cells, followed by incubation for at least 20 minutes at room temperature in 5mM phosphate buffer (pH7.4-7.6), 5mM EDTA, 0.2mM phenazine methosulfate, 50mM succinic acid, 1.5mM nitro blue (adjusted to pH 7.6 and filtered). Cells were then rinsed in 1x phosphate buffer, then 1x PBS, then water, and mounted in gelatin.

Supplementary references.

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