

Supplementary Information

Nandrolone induces a stem cell-like phenotype in human hepatocarcinoma-derived cell line inhibiting mitochondrial respiratory activity

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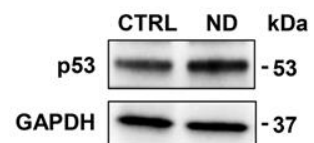
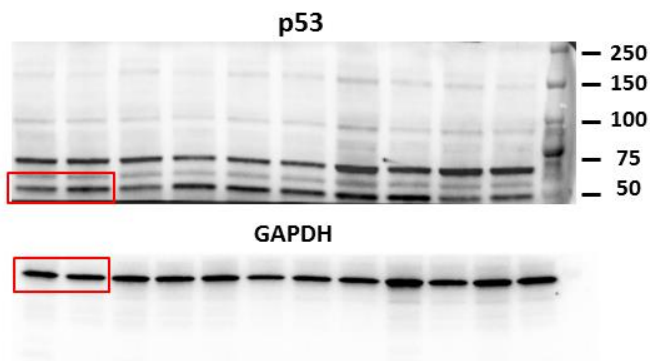
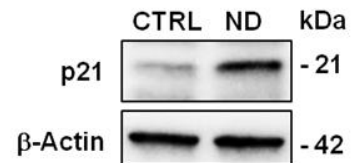
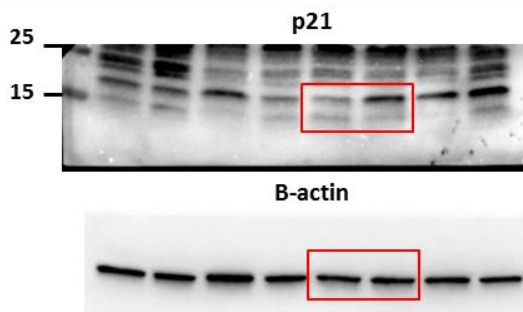
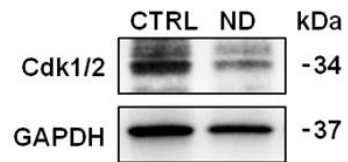
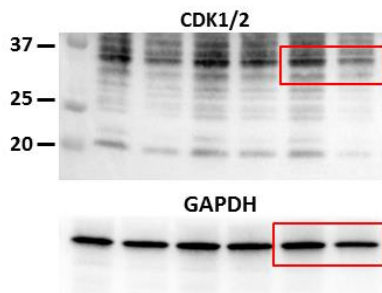
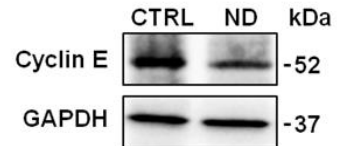
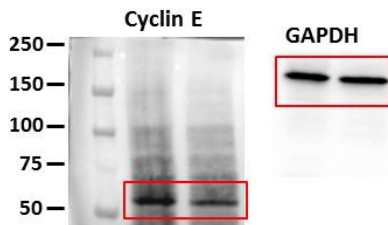
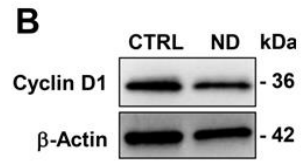
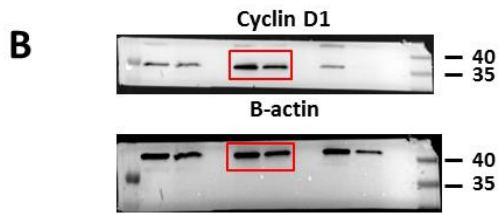
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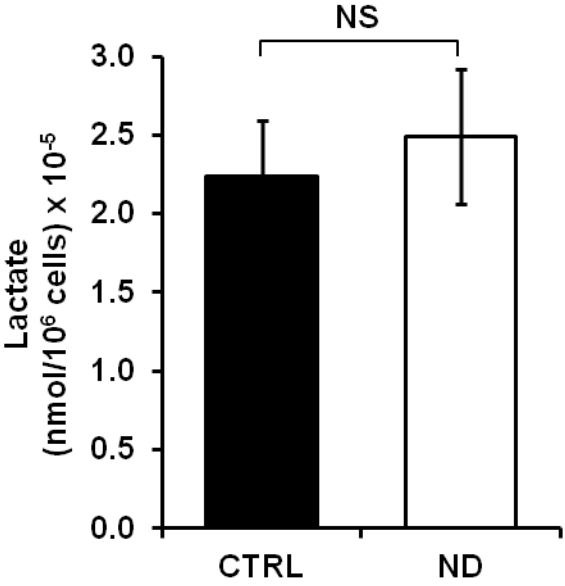
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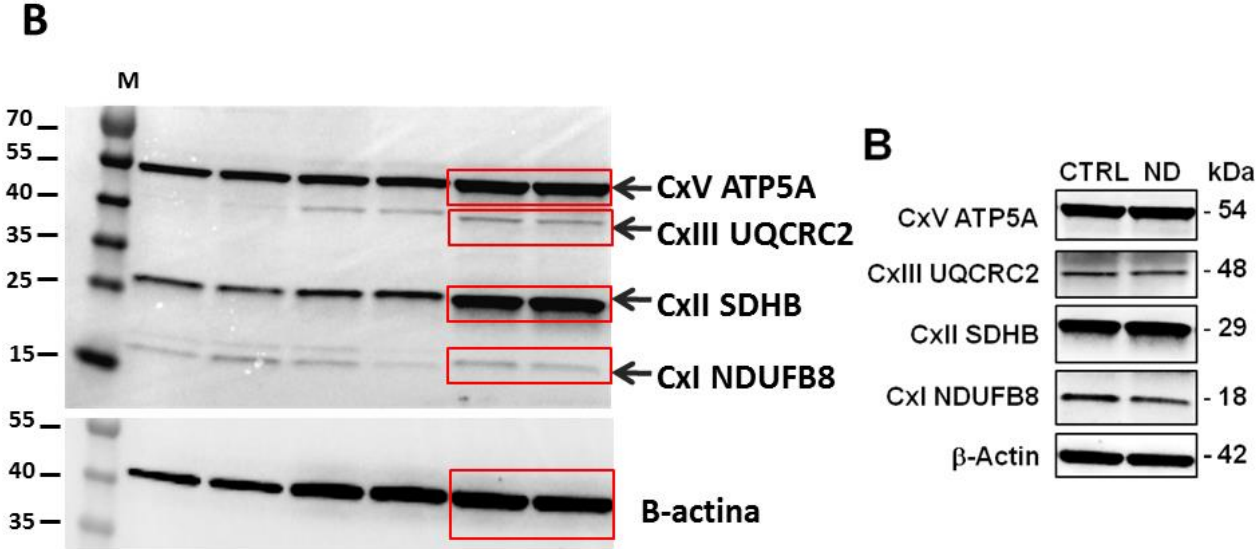
Supplementary Figure S1. Full-length blots of Figure 2B



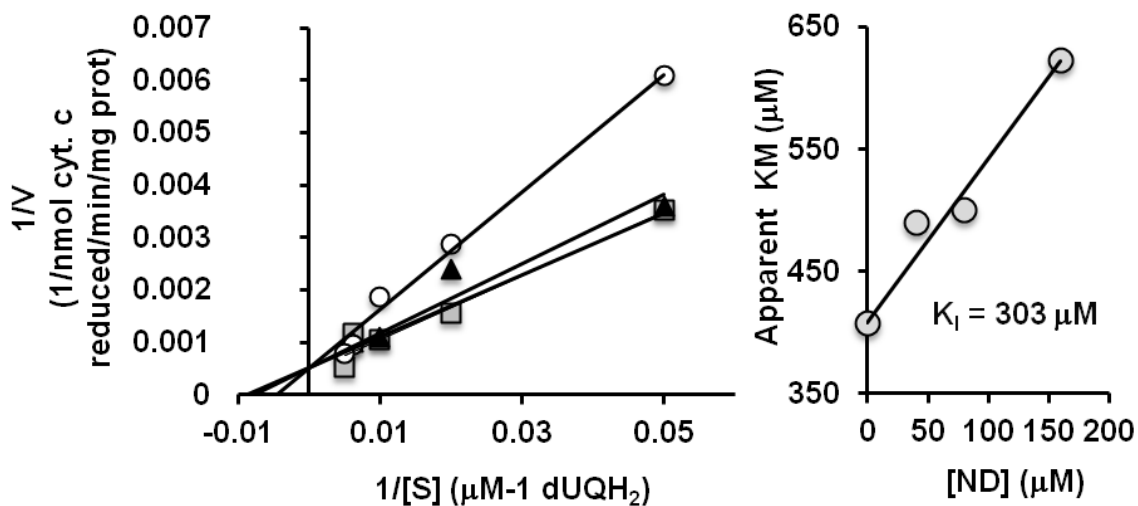
Supplementary Figure S2. Effect of nandrolone treatment on lactate production in HepG2 cells. The bars are average \pm SEM of three independent experiment. See Materials and Methods for details.



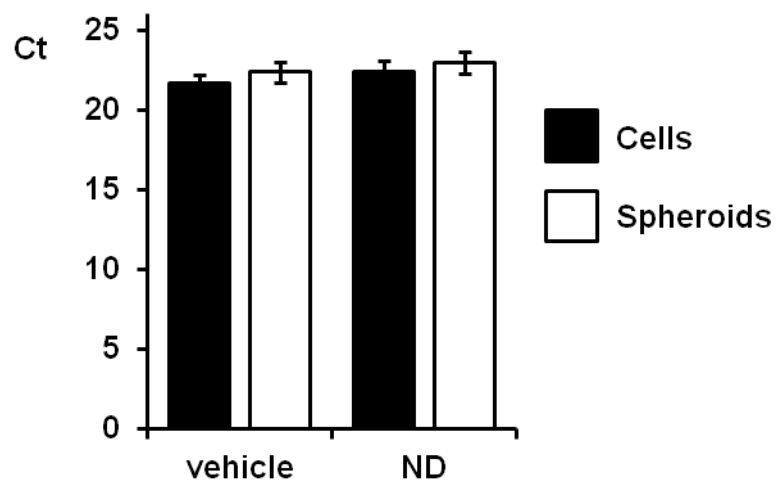
Supplementary Figure S3. Full-length blots of Figure 4B



Supplementary Figure S4. Evaluation of Nandrolone effect on the kinetic parameters of Ubiquinone:cytochrome c oxidoreductase (CxIII). Left graph: Lineweaver-Burk (L/B) plot illustrating inhibition of CxIII inhibition tested (in absence or) in the presence of different concentrations of ND as follows: 160 μM (○), 80 μM (▲), 40 μM (■). Graph on the right: secondary plot of slope against [ND] to determine the K_i (303 μM). The inhibition is competitive and the slopes were calculated from the L/B plot. CxIII activity was assayed in mitochondria isolated from beef heart by differential centrifugation as previously described [Srima R. et al. Plos One 12, e0188683 (2017)]. Mitochondria (1.0 mg prot/ml) were suspended in 0.25 M sucrose, 5 mM Hepes, 1 mM EDTA, 5 mM MgCl_2 and the kinetic parameters of CxIII were assessed spectrophotometrically (in the presence of 1.0 $\mu\text{g/ml}$ rotenone *plus* 2 mM KCN) by following the initial rate of 50 μM ferri-cytochrome c reduction ($\epsilon_{550\text{nm}} = 21.1 \text{ mM}^{-1} \text{ cm}^{-1}$) elicited by graded concentrations of dUQH₂ (20, 50, 100, 200, 300 μM) as electron donor in the absence or presence of graded concentrations of nandrolone (40, 80, 160, μM). The rates were corrected for the residual cytochrome c reduction in the presence of 1 $\mu\text{g/ml}$ antimycin A. The attained data points were plotted as double reciprocal plot and Dixon plot and linearly fitted by least square analysis using EnzFitter (Biosoft).



Supplementary Figure S5. Effect of nandrolone treatment (ND) on the expression of the housekeeping gene GAPDH in HepG2 cells and derived spheroids. Bars in the histogram are averages \pm SD of at least 6 independent assays under each condition and show the cycle threshold (Ct) of the RT-PCR analysis of the GAPDH expression (see Materials and Methods of the main text and Supplementary Table 1 for details). Black bars show the effect on cultured HepG2 cells of 72 h treatment with vehicle (ethanol) or 80 μ M nandrolone. White bars show the effect of nandrolone on forming spheroid with 80 μ M of the drug supplemented to the cell suspension at the beginning of the spheroid formation protocol (see Materials and Methods of the main text for details) and assessed after 72 h.



Supplementary Table 1. Features of the primers used for real-time polymerase chain reaction

| Gene | Organism | T_{ann} (°C) | Length (bp) | NCBI Ref Seq or sequence |
|-------------|----------------------|-----------------------------|--------------------|---|
| Nanog | Mouse (Mus musculus) | 55 | 114 | NM_028016 |
| Nestin | Mouse (Mus musculus) | 60 | 81 | NM_016701 |
| Myc | Mouse (Mus musculus) | 60 | 60 | NM_001177352 |
| Lin28 | Mouse (Mus musculus) | 55 | 116 | NM_145833 |
| Sox17 | Mouse (Mus musculus) | 60 | 77 | NM_011441 |
| Klf4 | Mouse (Mus musculus) | 60 | 66 | NM_010637 |
| Rn18S | Mouse (Mus musculus) | 60 | 97 | Forward: acaggattgacagattga Reverse: tatcggaattaaccagaca |
| Nanog | Homo sapiens | 55 | 90 | NM_024865 |
| Myc | Homo sapiens | 60 | 129 | NM_002467 |
| Lin28 | Homo sapiens | 55 | 126 | NM_024674 |
| Sox2 | Homo sapiens | 60 | 64 | NM_003106 |
| Klf4 | Homo sapiens | 60 | 72 | NM_004235 |
| Gapdh | Homo sapiens | 60 | 135 | Forward: aggctgagaacgggaagc Reverse: ccatggtggtgaagacg |