Supplementary Materials

Proteomic and Bioinformatic Investigation of Altered Pathways in Neuroglobin-Deficient Breast Cancer Cells

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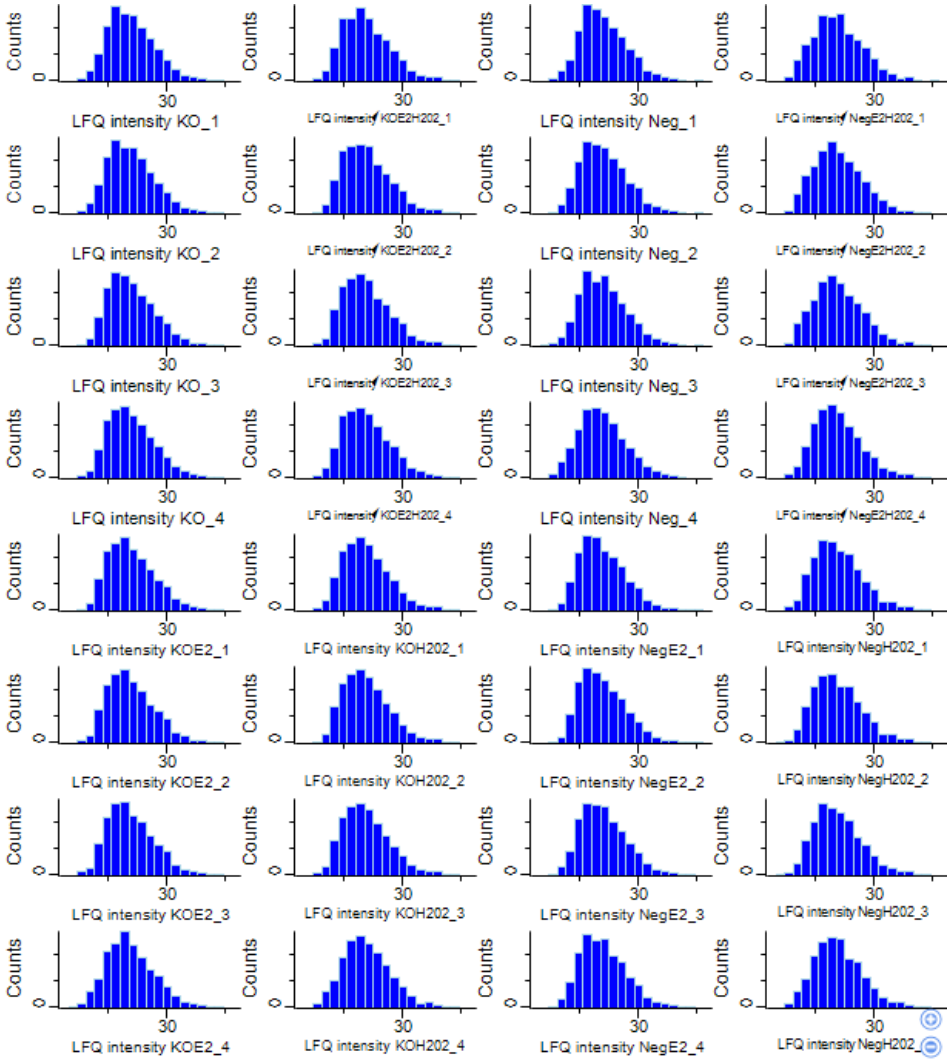
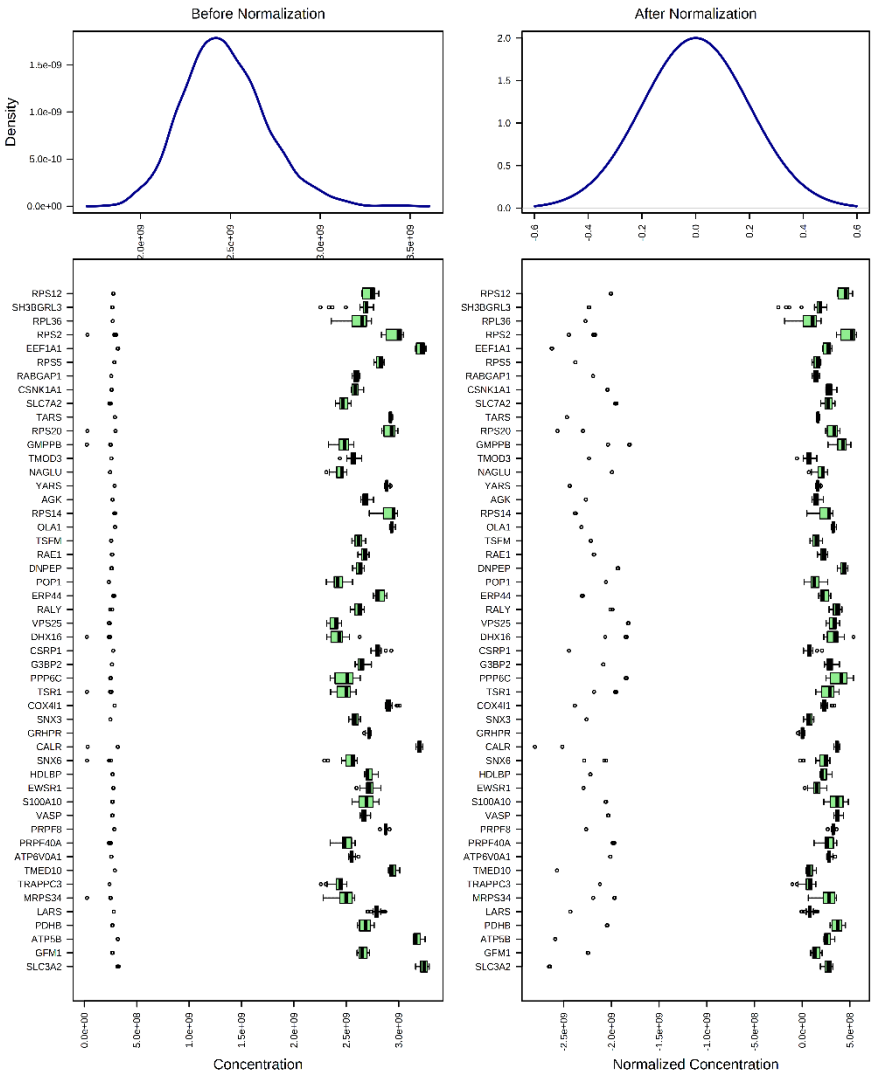
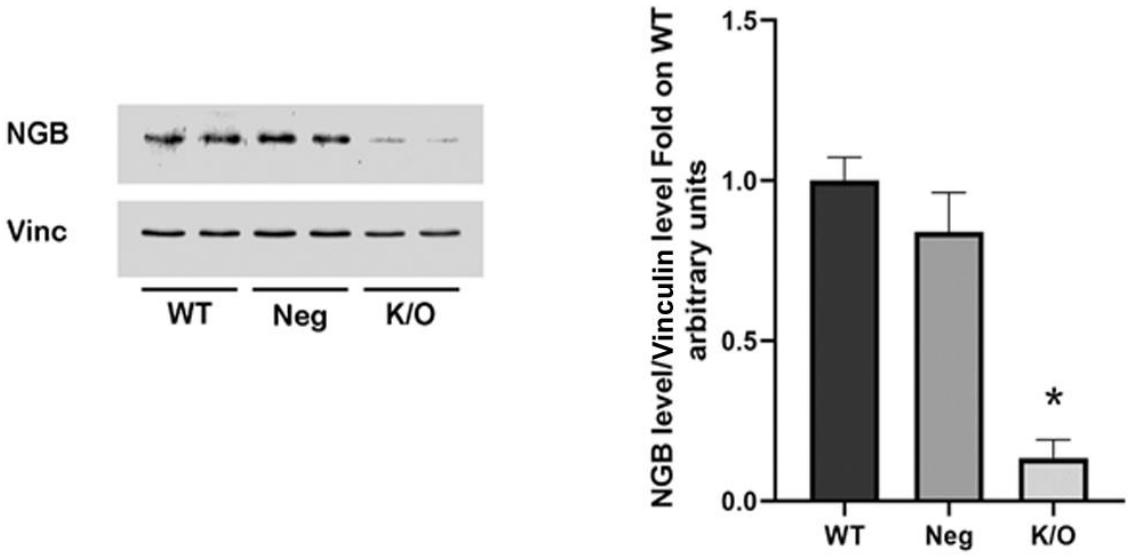


(a)



(b)

Figure S1. Statistic distribution of proteomics data. **(a)** Normal distribution of LFQ values of each LC-MS/MS run was evaluated with Perseus software plotting LFQ intensities as histogram bars; the plot for each biological replicate/condition/treatment is shown. **(b)** Normal distribution of data was adjusted by mean-centered scaling within MetaboAnalyst 4.0 software.



Scheme S2. NGB levels in CRISPR/Cas9 knockout MCF-7 cells. Representative Western Blot (left panel) and densitometric analysis (right panel) of NGB level in Wild Type (WT), Negative (Neg) and NGB-KO (K/O) MCF-7 cells. The levels of NGB proteins were normalized by comparison with vinculin levels. Western blot analysis was performed as previously described (*Solar Fernandez, V.; Cipolletti, M.; Ascenzi, P.; Marino, M.; Fiocchetti, M. Neuroglobin As Key Mediator in the 17β-Estradiol-Induced Antioxidant Cell Response to Oxidative Stress. Antioxid. Redox Signal.* ***2020****, 32, 217–227, doi:10.1089/ars.2019.7870*). Cells were harvested and lysed with a buffer mix (50 mM HEPES at pH 7.5, 10% glycerol, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 1 mM EGTA) containing 0.70% (*w*/*v*) SDS. Fifteen μg of solubilized proteins were resolved by 13.5% SDS-PAGE transferred into a nitrocellulose membrane using the Trans-Blot Turbo Transfer System (Bio-Rad Laboratories, Hercules, CA, USA) for 7 min at 25V. The membrane was then blocked with 5% (*w*/*v*) BSA in 138mM NaCl, 25mM Tris, pH 8.0, and 0.1 (*w*/*v*) Tween-20 at 25 °C for 1 h, and then incubated overnight at 4 °C with anti-NGB antibody (final dilution 1:1000, MERK Millipore, Darmstadt, DE), and anti-Vinculin antibody (1:40000, MERCK Millipore, Darmstadt, DE) used to normalize the protein content. Antibody reactivity was observed with ECL chemiluminescence Western blotting detection reagent by using ChemiDoc XRS+ Imaging System (Bio-Rad Laboratories, Hercules, CA, USA). The densitometric analyses was performed by ImageJ software for Microsoft Windows (NIH, Bethesda, MD, USA). The statistical analysis was performed by Student's t-test with the PRISM 6.01 software system (GraphPad Software, Inc, San Diego, CA, USA) for Windows. Data are means ± SD of at least three different experiments. * = $p < 0.05$.