

Supporting Information

Bryonolic Acid Transcriptional Control of Anti-inflammatory and Antioxidant Genes in Macrophages in Vitro and in Vivo

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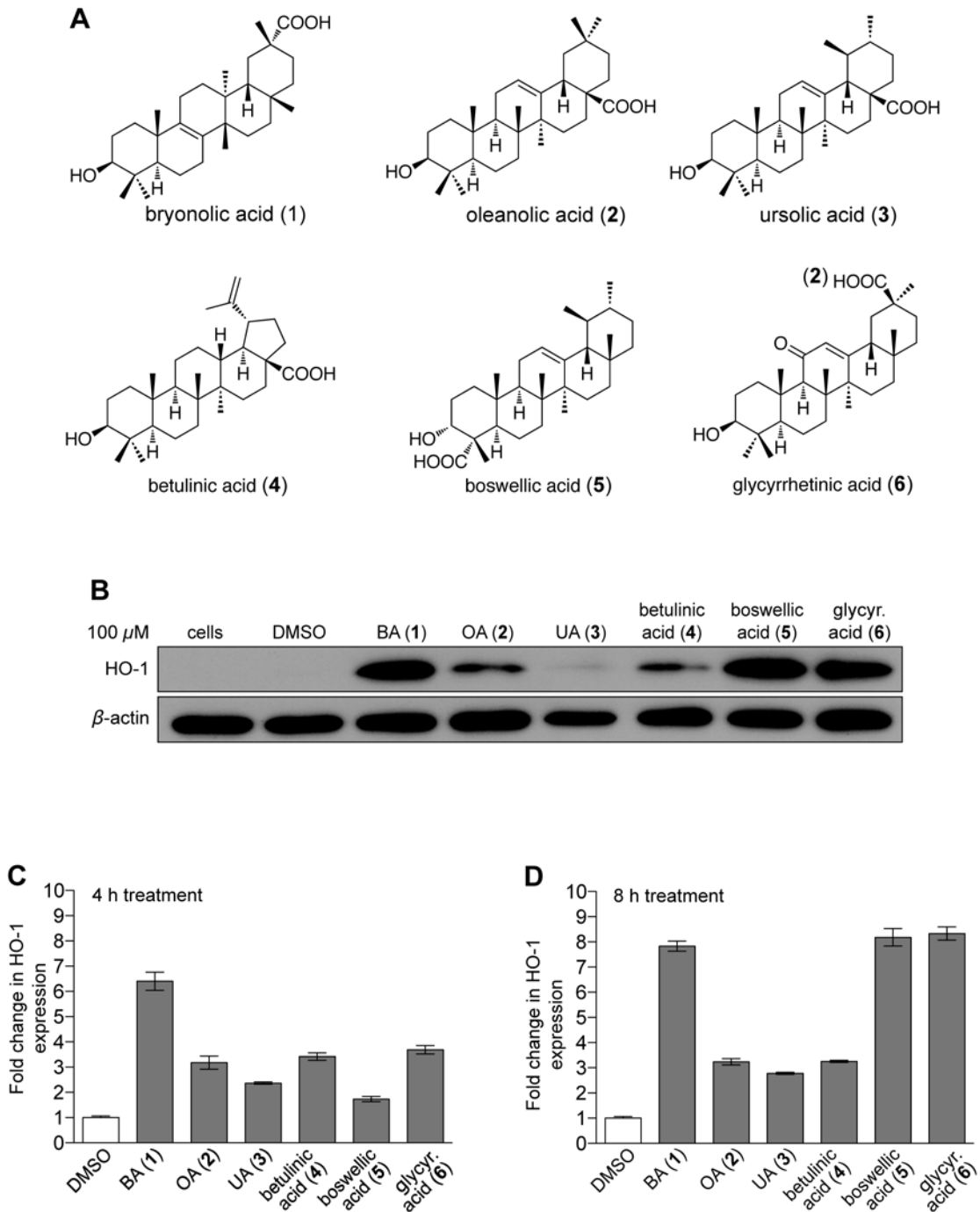


Figure S1. Comparative analyses of induction of HO-1 expression by triterpenoids (1-6). (A) Structurally similar triterpenoids were compared with bryonolic acid (BA) (1) for their ability to induce HO-1 expression. RAW 264.7 cells were treated with 100 μ M BA (1), oleanolic acid (OA) (2), ursolic acid (UA) (3), betulinic acid (4), boswellic acid (5), or glycyrrhetic acid (glycyrr. acid) (6). (B) HO-1 protein levels were quantified through immunoblot analyses in cells treated with triterpenoids (1-6) for 8 h. HO-1 mRNA levels were quantified via qRT-PCR in cells treated for 4 h (C) or 8 h (D).

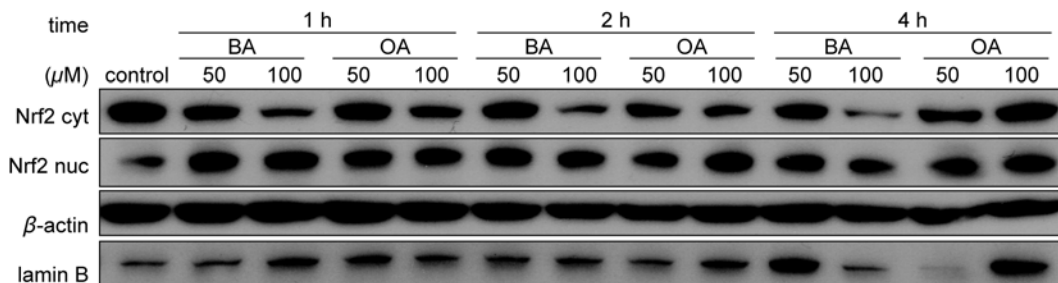


Figure S2. Bryonolic acid (BA) (1) induces translocation of Nrf2 into the nucleus. RAW 264.7 cells were treated with 50 μ M and 100 μ M BA (1) or oleanolic acid (OA) (2) at 1, 2 and 4 h and probed for nuclear and cytoplasmic Nrf2

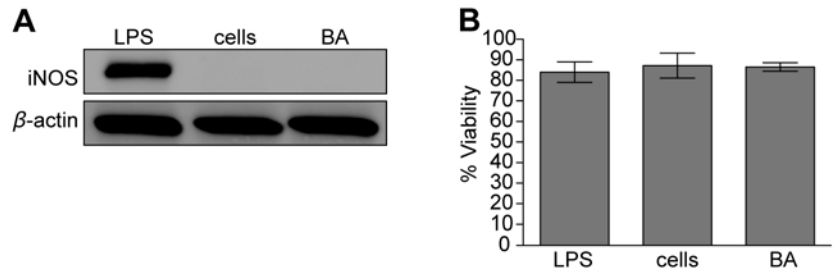


Figure S3. Positive and negative control for bryonolic acid (BA) (1)-treated cells. RAW 264.7 cells were treated with 5 ng/mL LPS or 100 μ M BA for 24 h. Immunoblot analysis of iNOS protein levels with β -actin as loading control (A) and cell viability measurement via tryphan blue exclusion test (B).

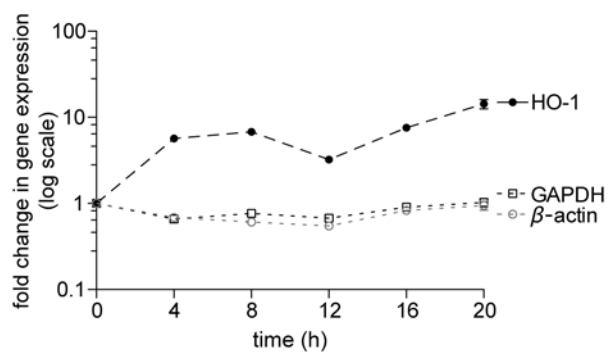


Figure S4. BA selectively regulates Nrf2 target genes. RAW 264.7 cells were treated with 100 μ M bryonolic acid (BA) (**1**) at different time points. qRT-PCR was performed to measure β -actin and GAPDH.