Supplemental Data

Edwards et al., The anti-inflammatory activity of curcumin is mediated by its oxidative metabolites

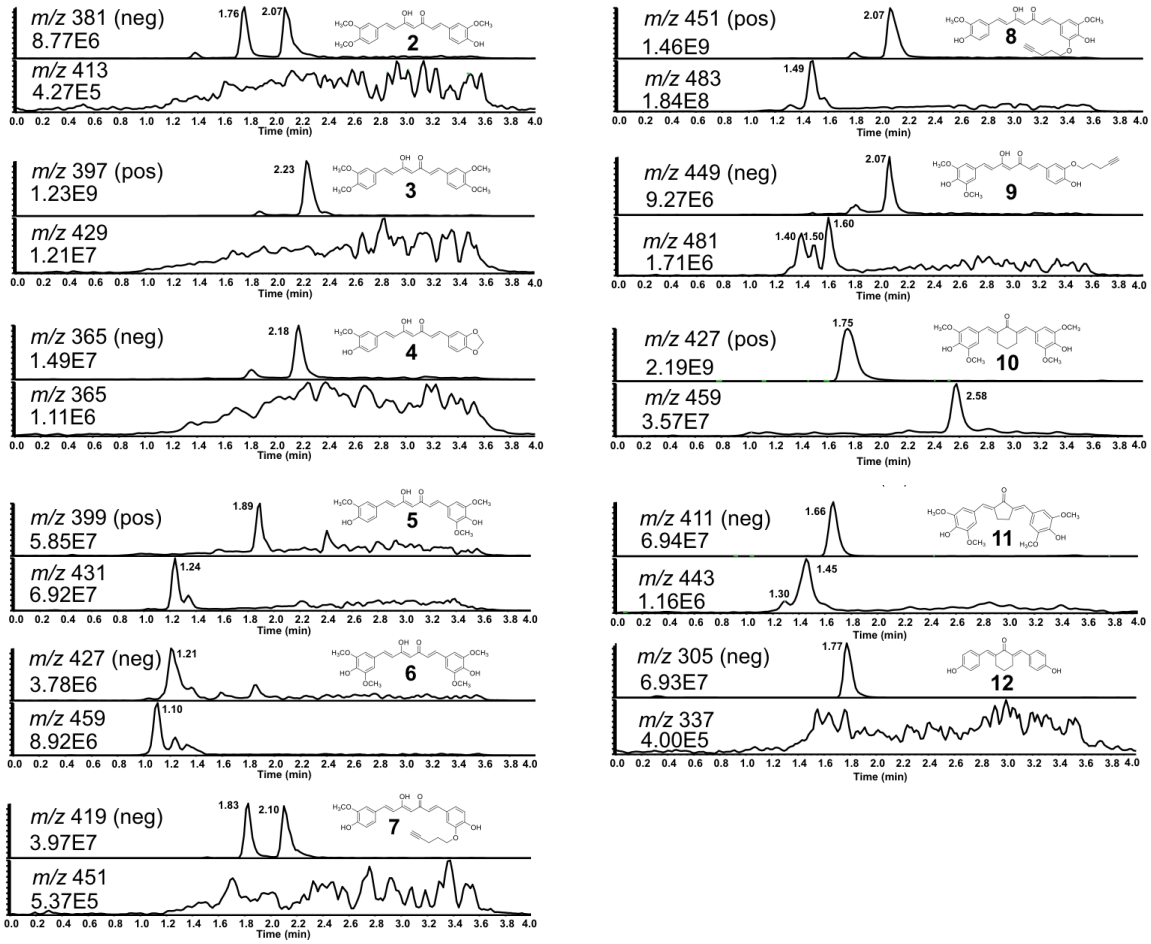

Fig. S1. LC-MS analysis of autoxidation reactions of compounds **2**-**12**. The top chromatogram for each compound shows the ion trace for the starting material and the bottom chromatogram the ion trace for the oxidation product (+ 32 amu). The compounds (50 μM) were incubated in 20 mM ammonium acetate buffer pH 7.5 for 20 min and extracted using Waters HLB cartridges. Samples were analyzed using a Thermo Vantage triple quadrupole mass spectrometer operated in positive or negative ion mode and coupled to a Waters Acquity UPLC system with a Waters symmetry Shield C18 3.5 μm column (2.1 x 100 mm) using a gradient of 5% to 95% acetonitrile in water/0.1% formic acid within 3 min and a flow rate of 0.4 μl/min.

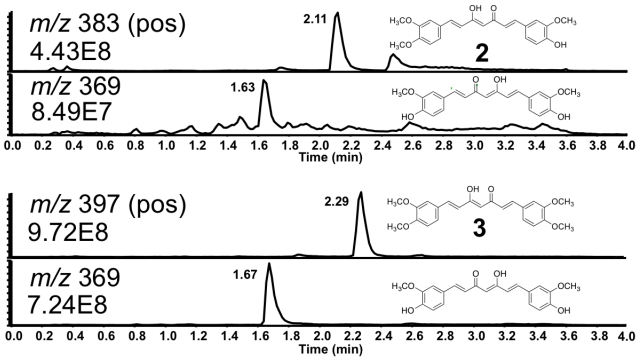

Fig. S2. Compounds **2** and **3** are metabolized to curcumin by RAW264.7 cells. LC-MS analysis of RAW264.7 cell supernatants treated with analogs **2** and **3**. The top chromatograms show the ion traces for analogs **2** (*m*/*z* 383) and **3** (*m*/*z* 397), respectively. The chromatograms below show ion traces for curcumin (*m*/*z* 369).

Samples were analyzed using a Thermo Vantage triple quadrupole mass spectrometer operated in positive ion mode and coupled to a Waters Acquity UPLC system with a Waters symmetry Shield C18 3.5 μm column (2.1 x 100 mm) using a gradient of 5% to 95% acetonitrile in water/0.1% formic acid within 3 min and a flow rate of 0.4 μl/min.

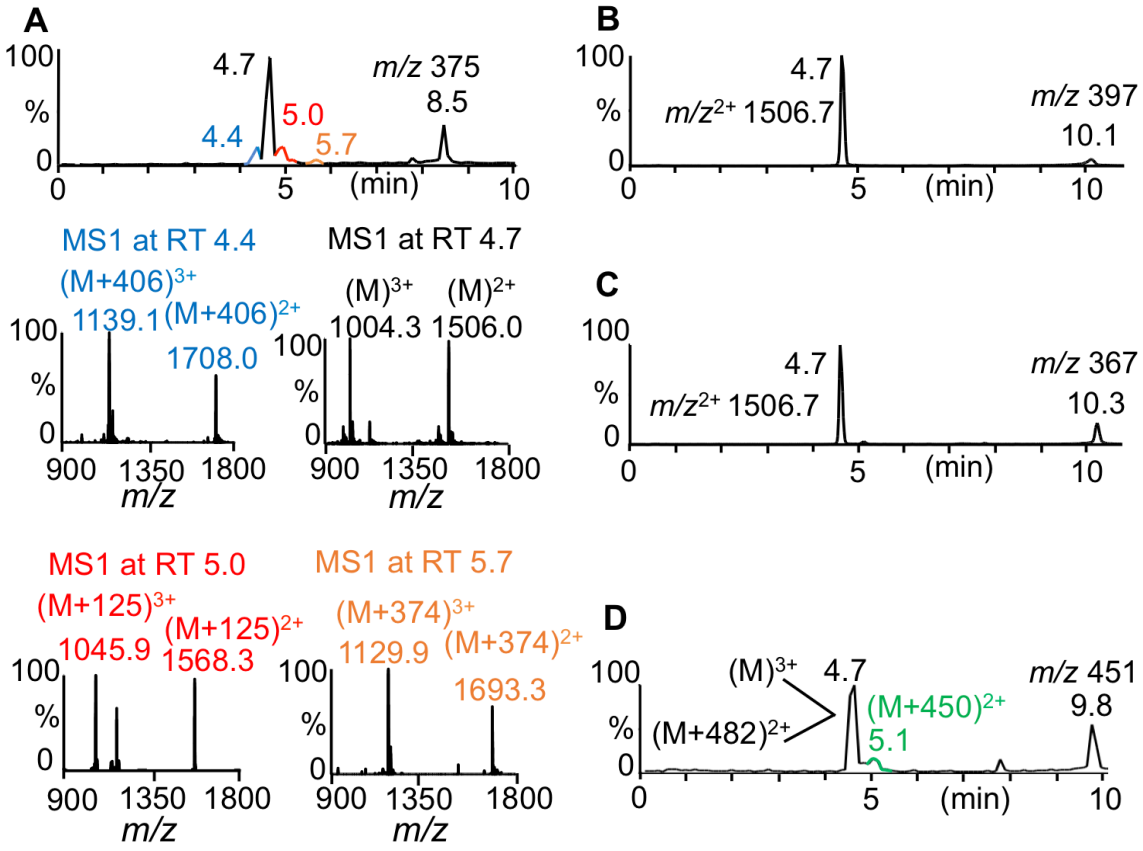

Fig. S3. Oxidative metabolites of curcumin covalently bind IKKβ. (A) Curcumin-d6 (50 μM) was autoxidized in the presence of a 27-amino acid peptide (10 μM) representing the activation domain of IKKβ for 1 h, and the mixture was analyzed by LC-MS. The MS1 spectra for the unmodified peptide (retention time 4.7 min) as well as peptide increased by 406 amu (RT 4.4 min), 374 amu (RT 5.7 min), and 127 amu (RT 5.0 min) are shown. (B-D) LC-MS analysis of the 27-amino acid peptide (10 μM) incubated with compound **3** (B), compound **4** (C), and compound **8** (D).