1   **Supplemental File 2**
2
3
4   **Activation of Nrf2 in the Liver is Associated with Stress Resistance Mediated by**
5   **Suppression of the Growth Hormone-Regulated STAT5b Transcription Factor**

6   John Rooney, Keiyu Oshida[1, 2], Naresh Vasani[1], Beena Vallanat[1], Natalia Ryan[1], Brian N.
7   Chorley[1], Xuting Wang[3], Douglas A. Bell[3], Kai C. Wu[4], Lauren M. Aleksunes[5], Curtis D.
8   Klaassen[6], Thomas W. Kensler[7], and J. Christopher Corton[1,8]
9

10 [1]NHEERL, US-EPA, Research Triangle Park, NC 27711
11 [2]Present address: Toray Industries, Inc., Kanagawa, Japan
12 [3] Immunity, Inflammation, and Disease Laboratory, National Institute of Environmental Health
13   Sciences, Research Triangle Park, NC 27709
14 [4]University of Kansas Medical Center, Kansas City, KS 66160Present address: Janssen
15   Pharmaceuticals, Spring House, PA 19477
16 [5] Rutgers University, Ernest Mario School of Pharmacy, Department of Pharmacology and
17   Toxicology, Piscataway, NJ 08854
18 [6]University of Washington, Seattle, WA 98195
19 [7] Department of Pharmacology & Chemical Biology, School of Medicine, University of
20   Pittsburgh, Pittsburgh, Pennsylvania 15261 Department of Pharmacology and Molecular
21   Sciences, School of Medicine, The Johns Hopkins University, Baltimore, Maryland 21205
22

23 **Classification analysis of Nrf2 activation using machine learning algorithms.**
24
25   Nrf2 activation was predicted using 7 classification models as detailed in the Methods
26 section. To determine the pcontribution of comparisons between chemically-treated wild-type
27 and Nrf2-null samples, two training sets were used in the prediction models including the
28 samples from livers of wild-type and Nrf2-null mice treated with CDDO-Im (Yates et al., 2009)
29 and the same dataset excluding the control and treated Nrf2-null samples. The derived classifiers
30 of 175 and 92 probe sets, respectively were then used to predict Nrf2 activation of test samples.
31 An independent manually curated test set came from mice with known Nrf2 activation status.
32 The models using the wild-type and Nrf2-null samples in the training set had excellent sensitivity
33 (mean, 100%) but low specificity (specificity range, 30-94%; mean, 62%) (data not shown). The
34 models using only the wild-type samples as the training set had lower sensitivity compared to
35 using all samples (mean, 57%) but somewhat greater specificity (range, 71-84%; mean, 78%),
36 indicating that the wild-type vs. null comparison of CDDO-Im treatment contributed to improved
37 sensitivity in classification predictions. Because of the low specificity or sensitivity of the
38 models, none were thought to be adequate for predicting Nrf2 activation of additional samples.
39
40
41 **Reference**
42

43 Yates MS, Tran QT, Dolan PM, Osburn WO, Shin S, McCulloch CC, Silkworth JB, Taguchi K,
44   Yamamoto M, Williams CR, Liby KT, Sporn MB, Sutter TR, Kensler TW. Genetic versus
45   chemoprotective activation of Nrf2 signaling: overlapping yet distinct gene expression
46   profiles between Keap1 knockout and triterpenoid-treated mice. Carcinogenesis. 2009
47   Jun;30(6):1024-31.
48