Supplemental Methods

LC-MS/MS, LC-MS and LC-IMS-MS analyses. The focus of our analysis was peptide-centric due to the fact that our MS technologies made measurements at the peptide level. Redundant peptide identifications in the case of a single peptide matching multiple proteins (typically protein isoforms) were removed; therefore, each reported peptide matches back to a single protein. This approach works well for tissue homogenates, such as this, where less than 10% of the identified peptides were redundant in the UniProtKB Mus musculus (mouse) database: for other samples types (i.e. plasma), a large proportion of the identified peptides will be redundant and other approaches should be used. Significance differences between p53 KO and WT data was determined at the peptide level with a p-value & q-value <0.05 (Supp. Table 1 & 2). DAnTE generates p-values and estimates their q-values. The q-value of a test measures the proportion of false positives incurred (called the false discovery rate) when that particular test is called significant. A small subset of these peptides (~7%) were deemed significant from an "all or none comparison," where a peptide was detected in ≥ 5 datasets of one sample type and not detected in the other sample type (these peptides were not seen in one of the sample types because they were (1) below instrument detection limits or (2) they were not expressed). To make sure that significant peptides matching back to the same protein were showing the same directionality/expression pattern, we rolled them together and redid our statistical tests (Supp. Table 1 & 2). Lastly, we wanted to make sure these

significant peptides were representative of the overall protein behavior. The goal of this study was to characterized peptides with relative abundance changes between our $p53^{d/d}$ and $p53^{f/f}$ samples, therefore we wanted to exclude peptides which were showing intensity changes not related to abundance (i.e. posttranslational modifications). Supplemental Tables 3 & 4 depict the overall protein behavior obtained by rolling up all peptides values (regardless if they were significant) into a protein value. Peptide amino acid sequences determine how well they can be characterized by mass spectrometry (i.e. some peptides do not ionize well); therefore even within the same protein not all the peptides will show the same level of significant change. Supplemental Table 5 contains the final IMS-MS and Velos filtered results, all quantitative information, and functional categorization of each protein. Proteins reported in this manuscript were to be identified by at least 2 peptides, but cases when the significant peptide identified was the only peptide identified are listed in Supplemental Table 5, where they are denoted with grey text. Protein functionality was inferred from UniProtKB (http://www.uniprot.org, links for each protein can be found in Supplemental Table 5) GO annotation (Supplemental Table 6) and KEGG information (Supplemental Table 7-9).