Supporting Information

Data-independent acquisition mass spectrometry to quantify protein levels in FFPE tumor biopsies for molecular diagnostics

Yeoun Jin Kim*, [1], Steve MM Sweet[1], Jarrett D Egertson[3], Andrew J Sedgewick[2], Sunghee Woo[1], Wei-li Liao[1], Gennifer E Merrihew[3], Brian C Searle[3], Charlie Vaske[2], Robert Heaton[1], Michael J MacCoss[3], Todd Hembrough[1]

[1]NantOmics, 9600 Medical Center Dr, Rockville, MD 20850, [2]NantOmics, 2919 Mission St, Santa Cruz, CA 95060, [3]University of Washington, Department of Genome Sciences, 3720 15th Avenue NE, Seattle, WA 98195.

Supporting Fig S-1. An example of tissue images before and after microdissection (sample #8 was used in this figure). A. Hematoxylin and eosin (H&E) stained tissue section. B. Hemtoxylin stained tissue section after markup based on the H&E image and before microdissection. C. Hemtoxylin stained tissue section after microdissection.

Supporting Fig S-2. Standard curve showing linear response for EGFR peptide, IPLENLQIIR. A 14 point curve was generated by spiking 50 to 25,000 amol into a *Pyrococcus furiosus* background, with seven replicates at each point. Error bars show standard deviation.

Supporting Fig S-3. Standard curve showing linear response for TUBB3 peptide, ISVYYNEASSHK. An 8 point curve was generated by spiking 250 to 25,000 amol into a *Pyrococcus furiosus* background, with six replicates at each point. Error bars show standard deviation.

Supporting Fig S-4. Hierarchical clustering of 3,713 protein groups and 12 samples. Clustering carried out in Perseus v1.6.0.7. (Euclidean distance; average linkage; pre-process with k-means (300 clusters; maximum 10 iterations; 1 restart).

Supporting Fig S-5. Histogram of mRNA-protein correlations for each of 3,429 genes across twelve patients.

Supporting Table S-1. List of tumor biopsy samples.

Supporting Table S-2. SRM transitions

Supporting Table S-3. Comprehensive DIA results of 12 samples.

Supporting Table S-4. List of proteins identified from library (4,239).

Supporting Table S-5. List of non-redundant protein groups (3,713)

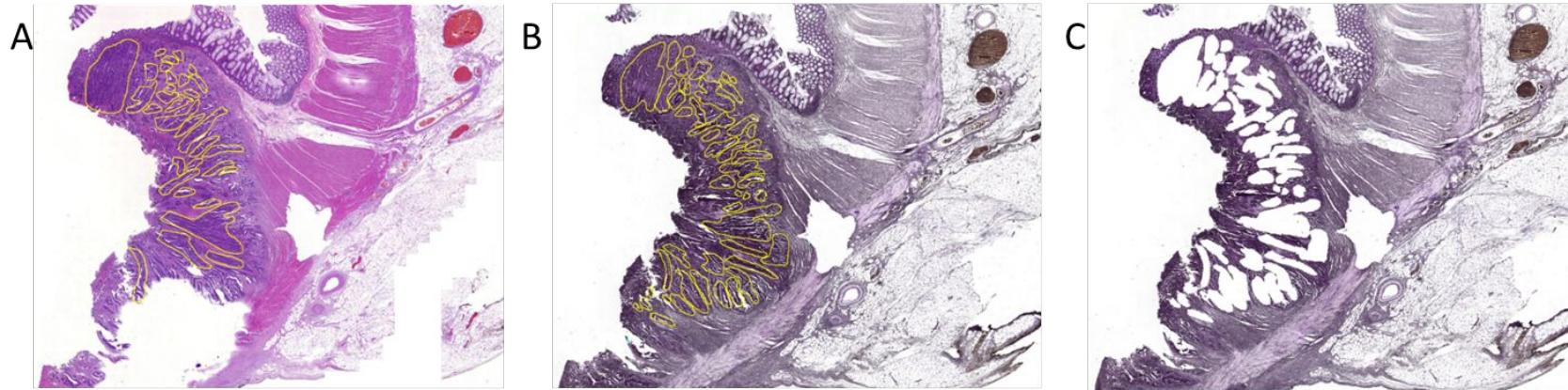Supporting Table S-6. List of proteins differentially expressed.

Fig S-1. An example of tissue images before and after microdissection (sample #8 was used in this figure). A. Hematoxylin and eosin (H&E) stained tissue section. B. Hemtoxylin stained tissue section after markup based on the H&E image and before microdissection. C. Hemtoxylin stained tissue section after microdissection.
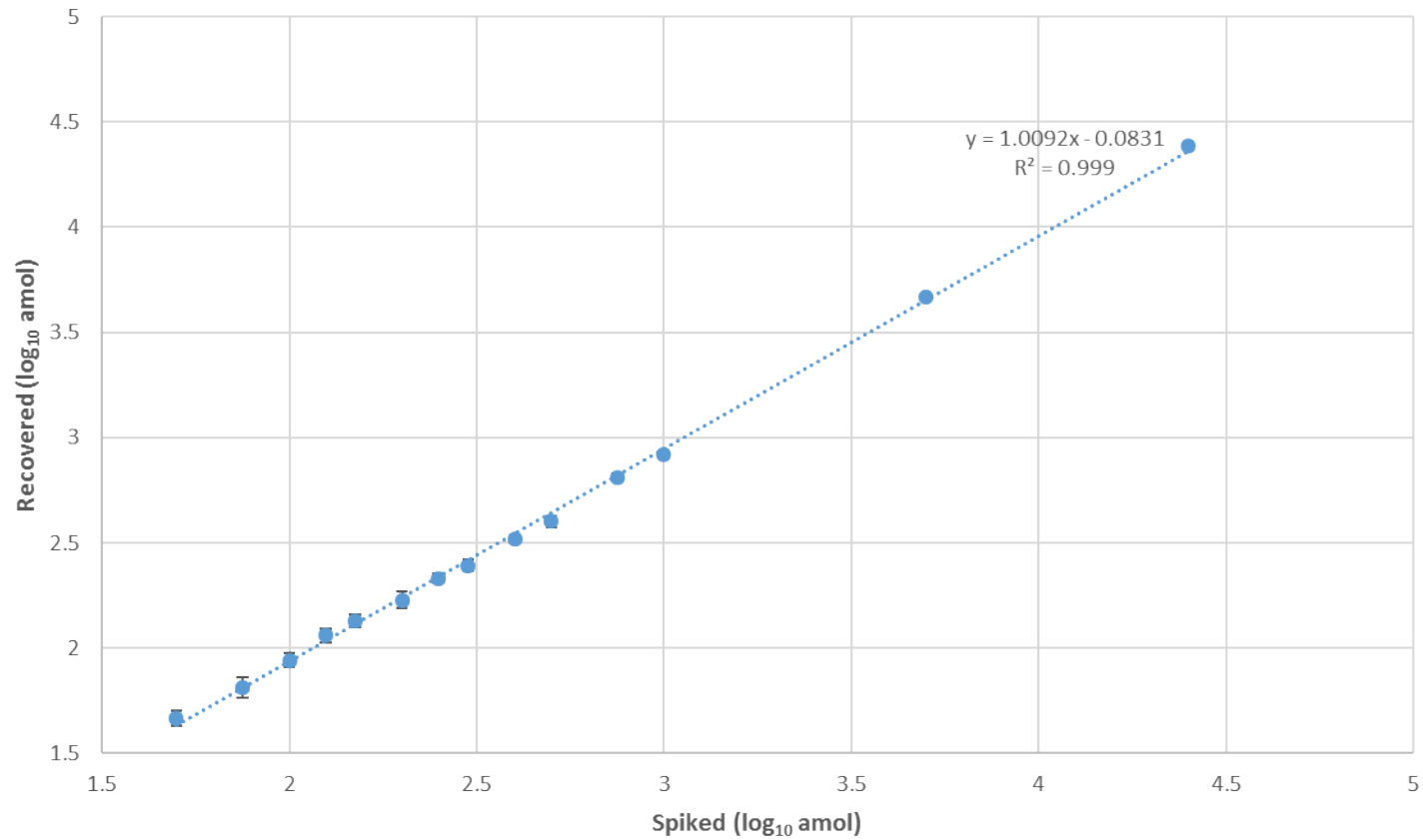
Fig S-2. Standard curve showing linear response for EGFR peptide, IPLENLQIIR. A 14 point curve was generated by spiking 50 to 25,000 amol into a *Pyrococcus furiosus* background, with seven replicates at each point. Error bars show standard deviation.
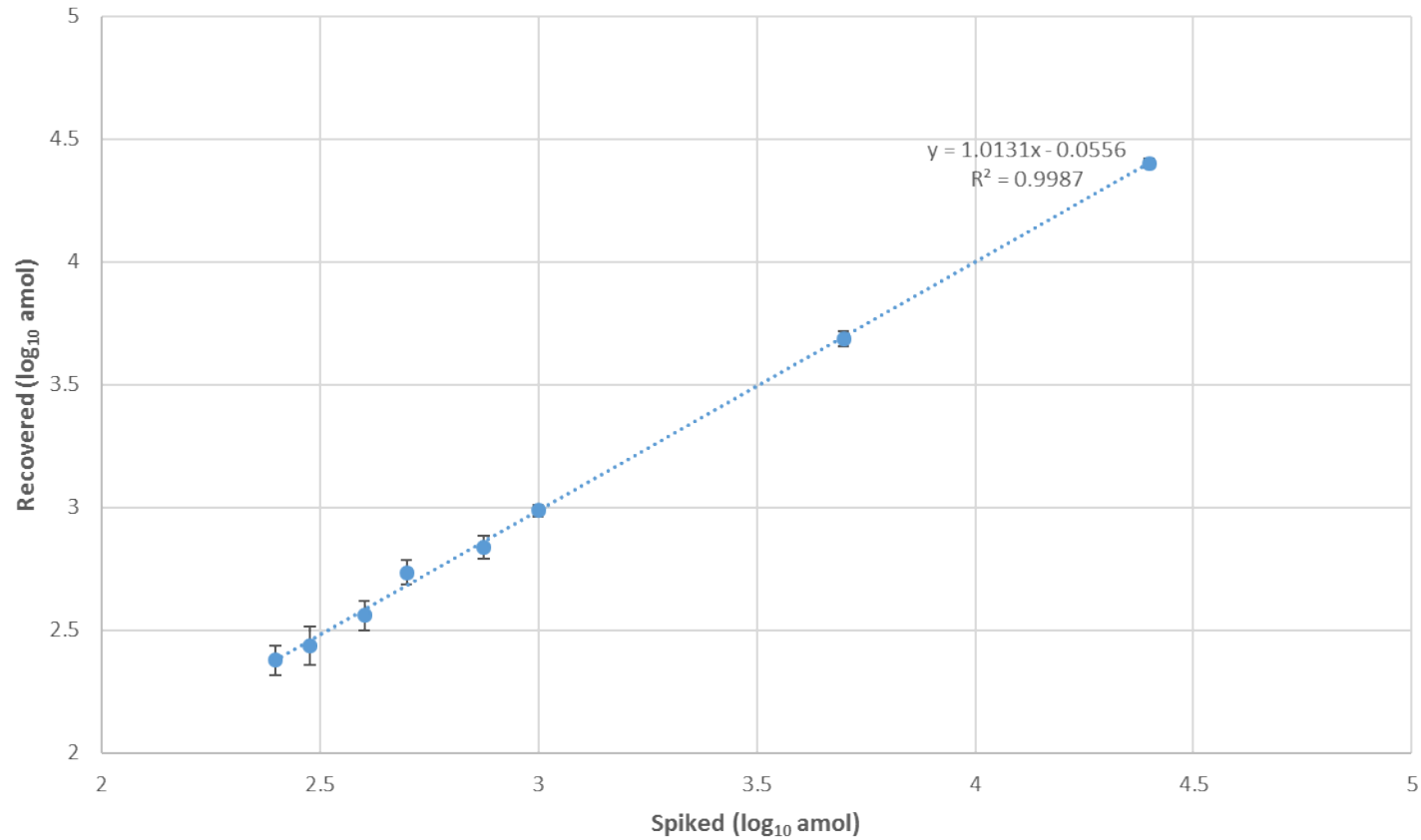
Fig S-3. Standard curve showing linear response for TUBB3 peptide, ISVYYNEASSHK. An 8 point curve was generated by spiking 250 to 25,000 amol into a *Pyrococcus furiosus* background, with six replicates at each point. Error bars show standard deviation.
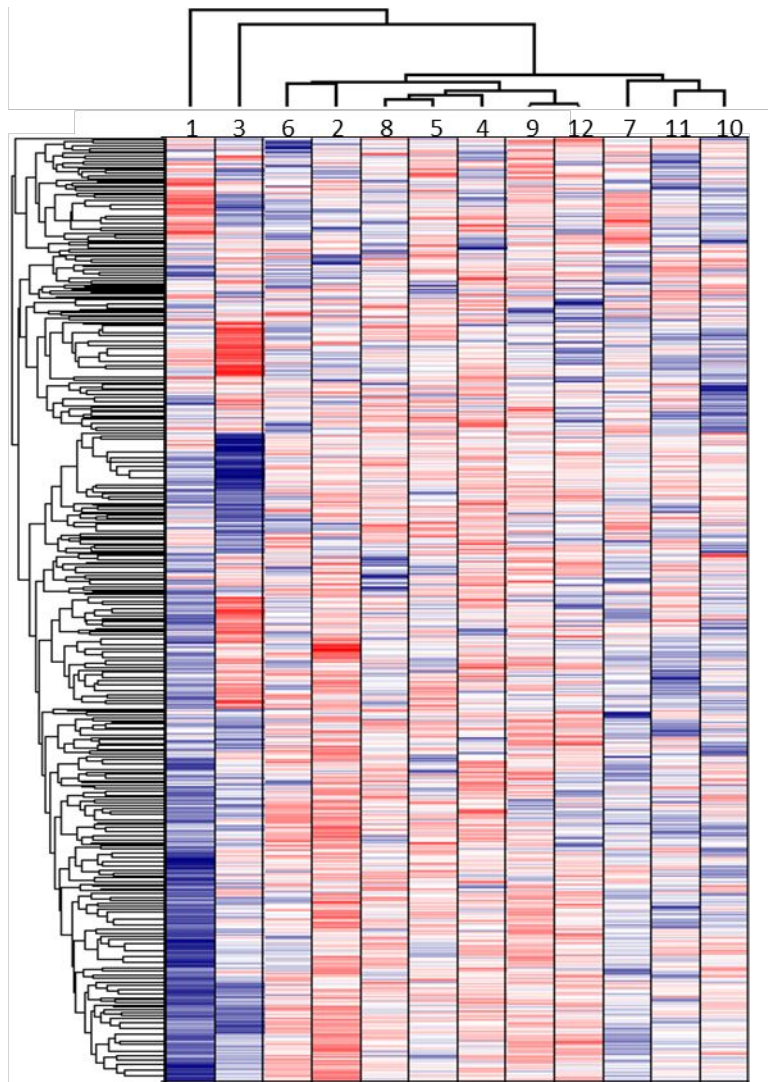
Fig S-4. Hierarchical clustering of 3,713 protein groups and 12 samples. Clustering carried out in Perseus v1.6.0.7. (Euclidean distance; average linkage; pre-process with k-means (300 clusters; maximum 10 iterations; 1 restart).
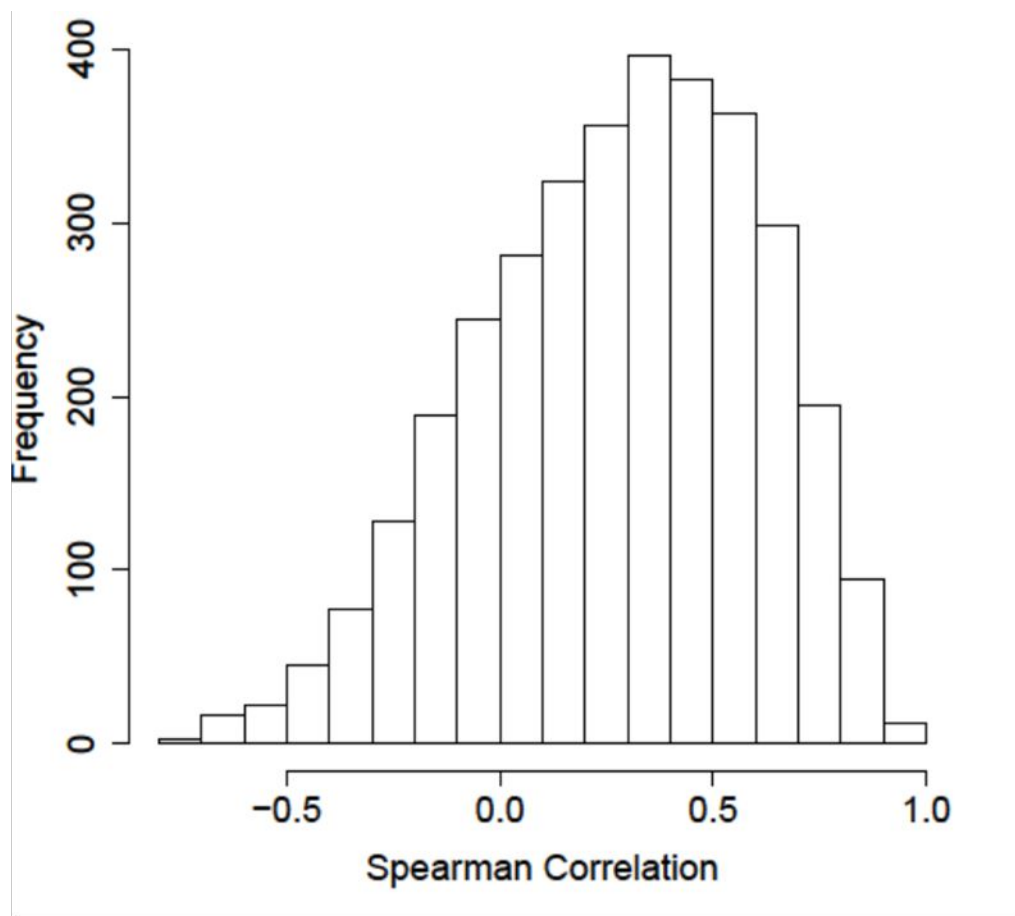
Fig S-5. Histogram of mRNA-protein correlations for each of 3,429 genes across twelve patients.