SUPPLEMENTARY FIGURES

Exome-capture RNA-sequencing of decade-old breast cancers and matched decalcified bone metastases

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Supplementary Figure 1. Expression correlation plots of ecRNA-seq sample sets.

Supplementary Figure 2. ecRNA-seq QC metrics for patient-matched cohort and bone metastasis clustering.

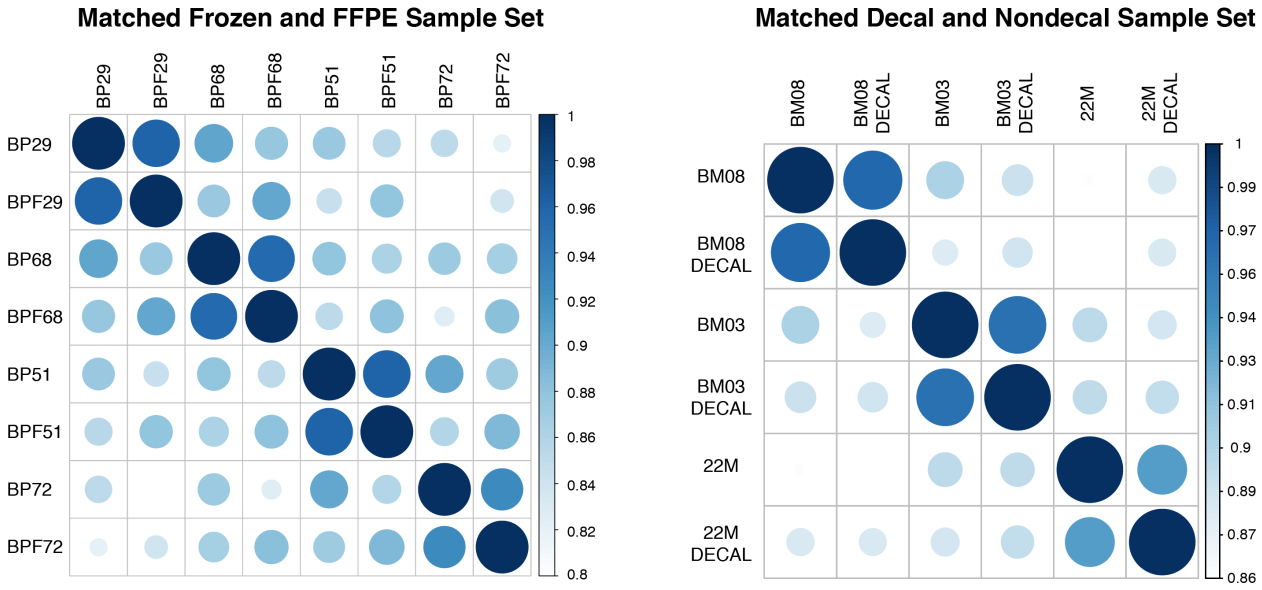
Supplementary Figure 3. tumorMatch: Proportion of shared variants (POSV) between samples in patient-matched cohort.

Supplementary Figure 4. Gene Ontology: Biological Processes (GO:BP) gene overlaps for differentially expressed gene sets.

Supplementary Figure 5. Case-specific expression fold-change distributions and expression alteration thresholds.

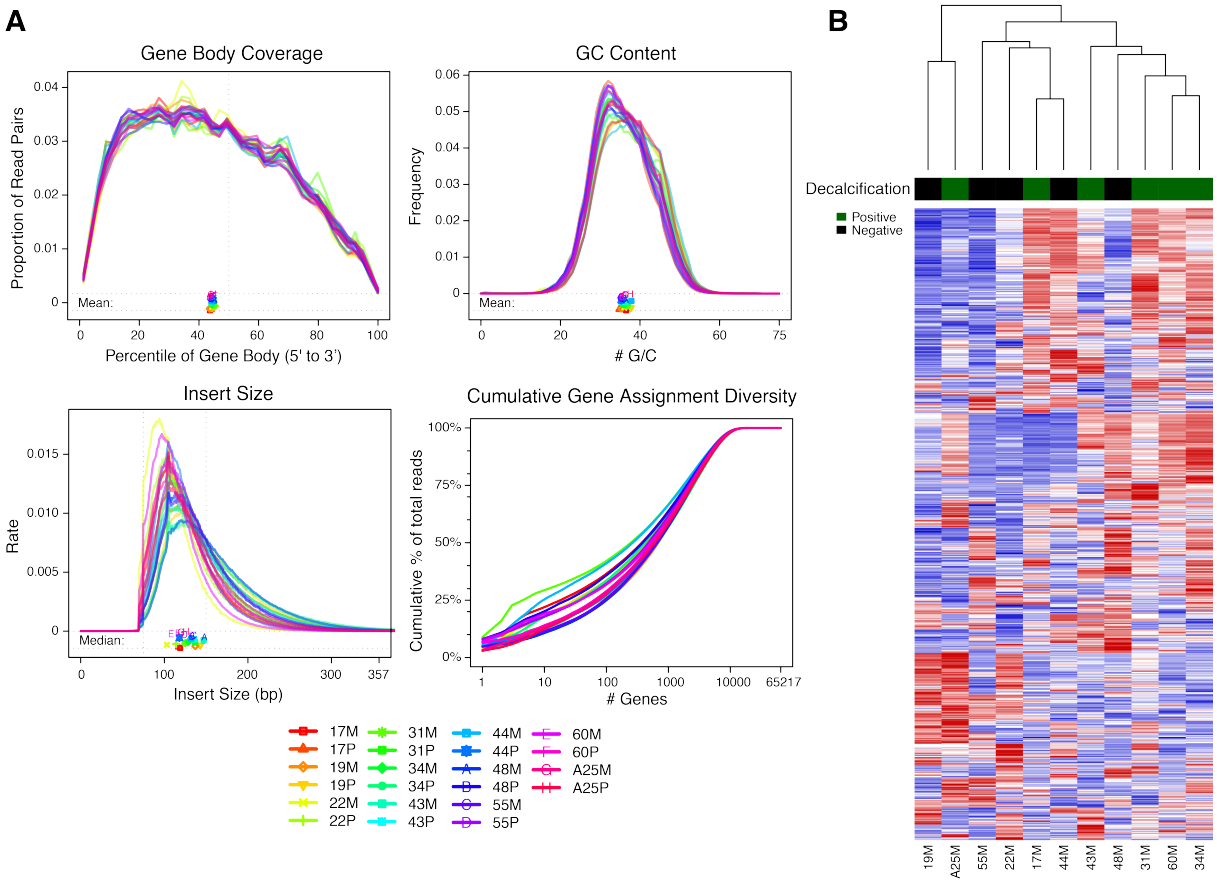
Supplementary Figure 6. Recurrent expression alterations in clinically actionable genes.

Supplementary Figure 1. Expression correlation matrices of ecRNA-seq sample sets



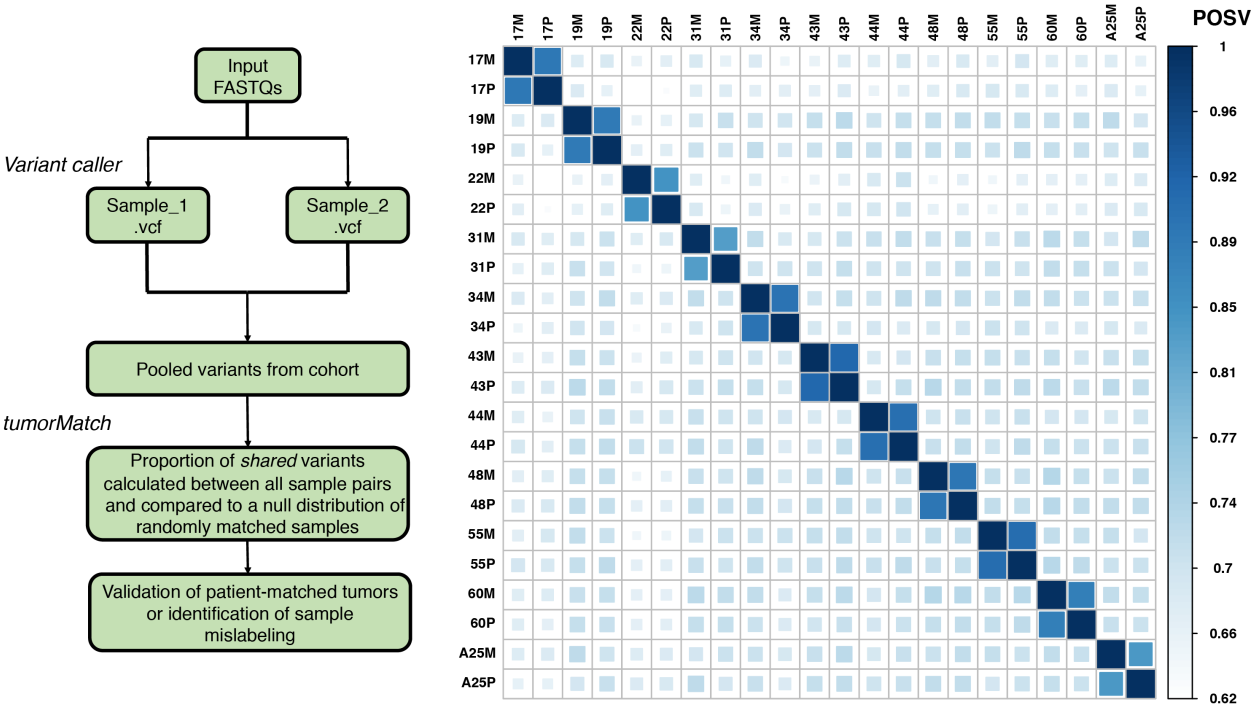
Supplementary Figure 1: **Expression correlation plots of ecRNA-seq sample sets.** Correlation plots of matched flash-frozen vs. FFPE and matched decalcified vs. nondecalcified sample sets. Both size and shade of color represent Pearson *r* correlations between all samples within each sample set; larger circles and darker blue colors represent higher correlations.

Supplementary Figure 2. ecRNA-seq QC metrics for patient-matched cohort and bone metastasis clustering.



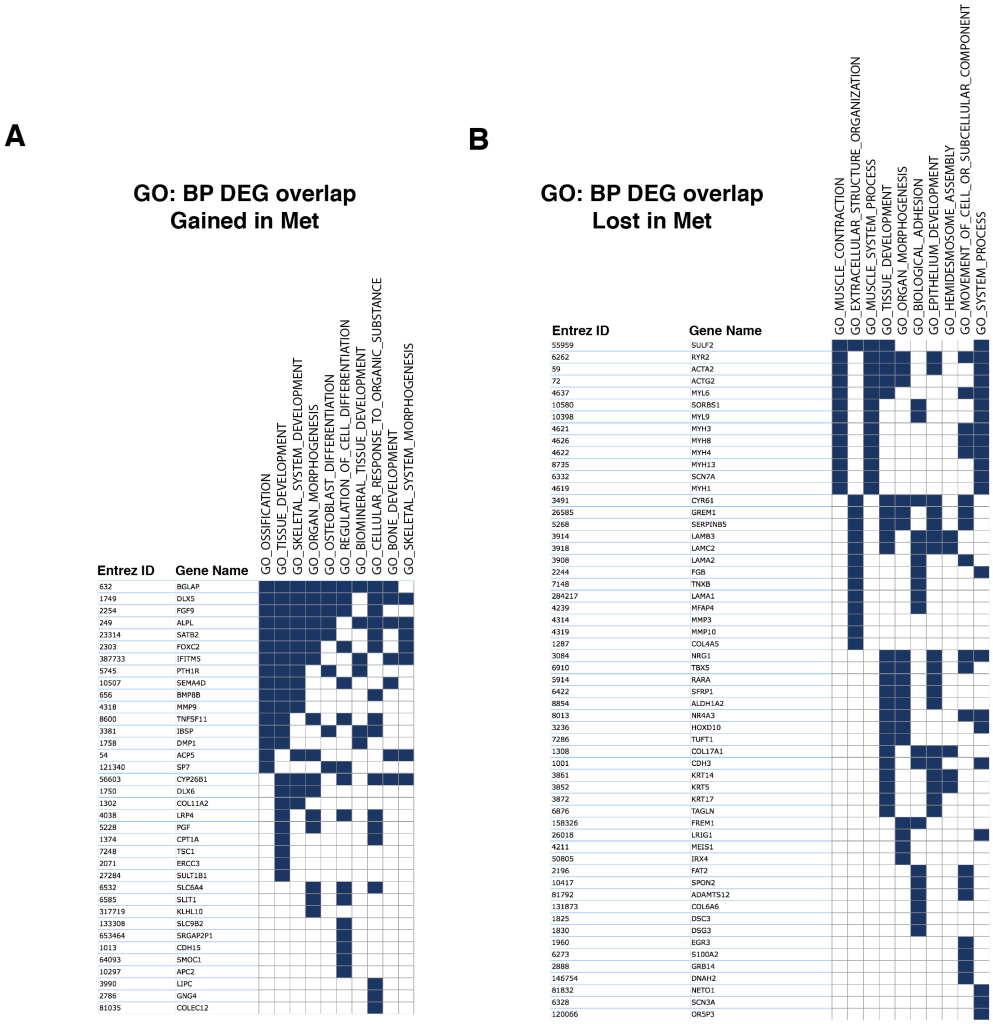
Supplementary Figure 2: ***(A)*** **ecRNA-seq QC metrics for patient-matched cohort and bone metastasis clustering. (A)** ecRNA-sequencing gene body coverage, GC content and insert size distributions along with gene assignment diversity assignments for all 22 tumors in patient-matched cohort. Each tumor is plotted with a different color, legend on right. **(B)** Unsupervised hierarchical clustering of bone metastases (n = 11) with decalcification status (green = positive, black = negative) indicated.

Supplementary Figure 3. tumorMatch: Proportion of shared variants (POSV) in patient-matched cohort



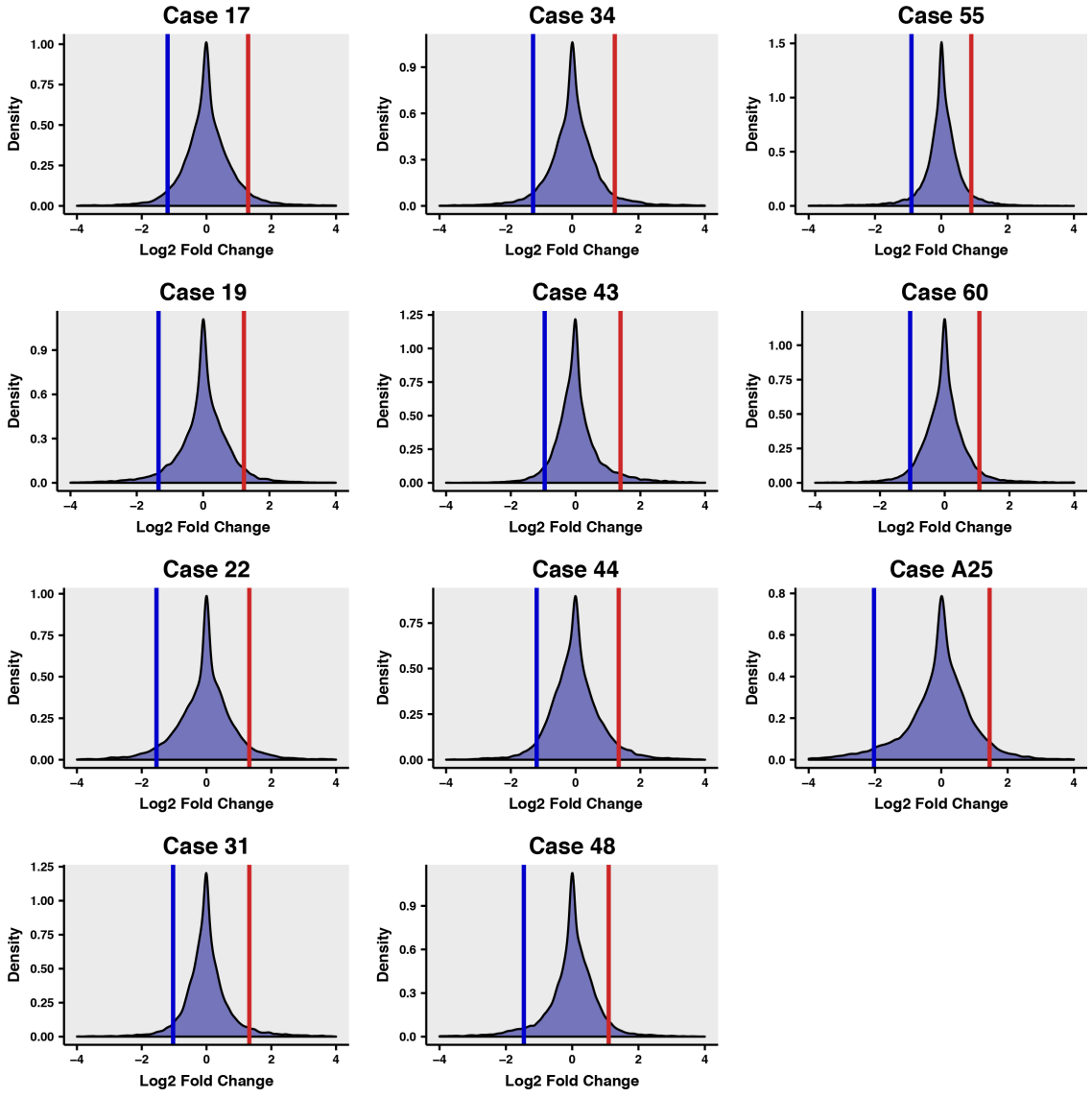
Supplementary Figure 3: **tumorMatch: Proportion of shared variants (POSV) between samples in patient-matched cohort.** Left, diagram outlining *tumorMatch* method which identifies patient-matched tumor specimens or sample mislabeling. Right, correlation plot showing proportion of shared variants between all 22 tumors in the cohort; bigger squares and darker blue color represents a higher proportion of shared variants (POSV) between two samples.

Supplementary Figure 4. GO: Biological process gene overlap for DEGs



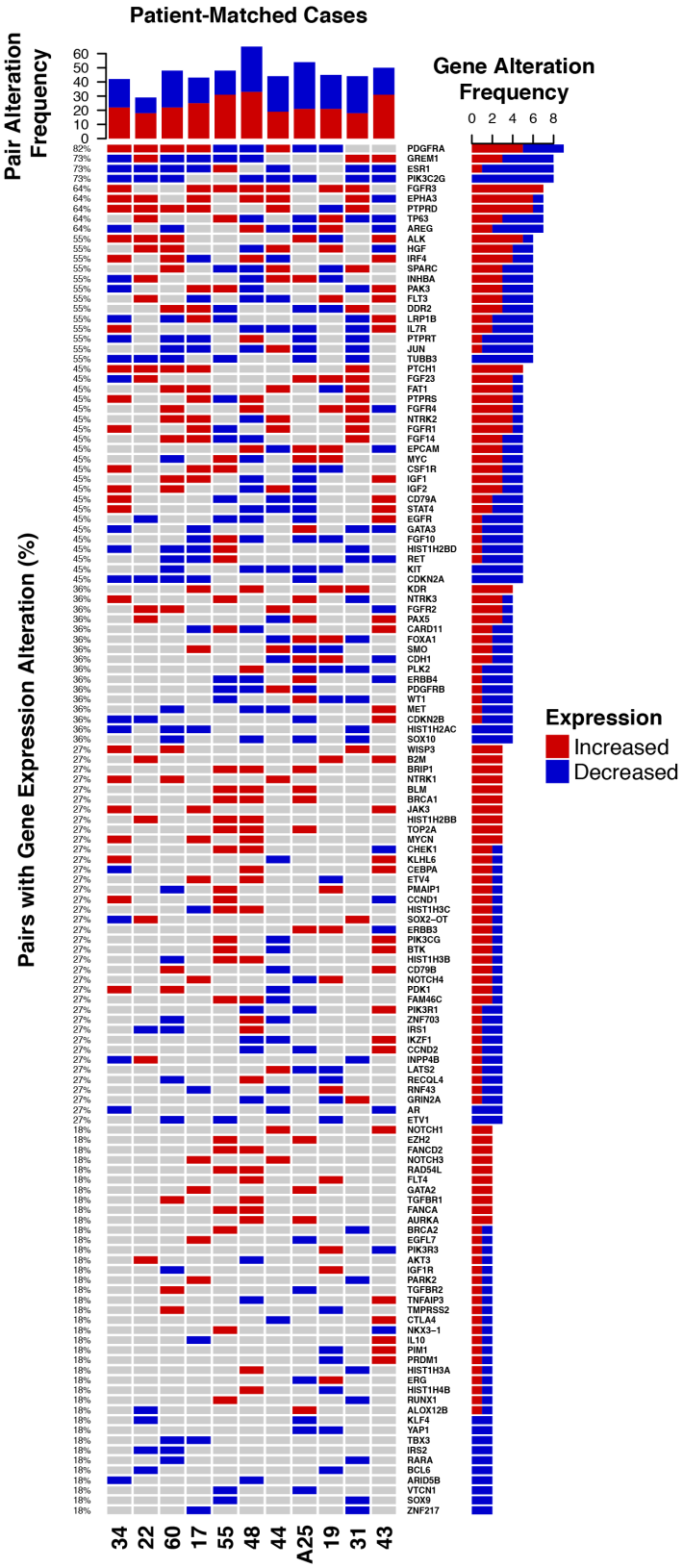
Supplementary Figure 4: **Gene Ontology: Biological Processes (GO:BP) gene overlaps for differentially expressed gene sets. (A)** GO:BP gene overlaps for genes with significant expression increases in bone metastases vs. patient-matched primaries. **(B)** GO:BP gene overlaps for genes with significant expression decreases in bone metastases vs. patient-matched primaries.

Supplementary Figure 5. Case-specific expression fold-change distributions and expression alteration thresholds



Supplementary Figure 5: **Case-specific expression fold-change distributions and expression alteration thresholds.** Fold-change density plots using log2normCPM values (Metastasis log2normCPM – Primary log2normCPM) for all genes. Expression alteration thresholds for significant expression loss (marked in blue, 5th percentile) and significant expression gain (marked in red, 95th percentile) shown for each of the 11 patient-matched cases.

Supplementary Figure 6. All recurrent expression alterations in clinically actionable genes



Supplementary Figure 6: **Recurrent expression alterations in clinically actionable genes.** Oncoprint plot showing all recurrent (> 1 case) expression alterations in bone metastases with each column representing a patient-matched case. Pair alteration frequencies, gene-specific expression alteration percentages and gene alteration frequency shown. Red tiles represent significant expression gains and blue tiles represent significant expression losses (as defined by case-specific expression alteration thresholds). Genes ranked by gene alteration frequency.