SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. Overview of approaches for identification of somatic mutations of all classes.

Supplementary Figure S2. Flow diagram of SNP analysis pipeline. Raw reads were mapped to the reference genome (hg19) using ELANDv2 and then FastQC was used to perform quality control checks on sequence data, followed by removing repeats, duplications and the reads that failed the platform quality check. SAMtools was then used to call SNPs and somatic SNPs. ANNOVAR was applied to filter known somatic SNP variants and to annotate novel somatic SNPs.

Supplementary Figure S3. Catalogue of somatic alterations in the metastatic melanoma that underwent WGS. Circos plot. Chromosomes are presented in circularly arranged ideograms, demarcated by a megabase (Mb) scale on the outer ring in a clockwise direction. Other tracks contain somatic mutations (from outside to inside): SNPs (purple dots, shown by density per Mb); structural variants - insertions (red triangles: validated; grey triangles: without validation) and structural variants - deletions (green triangles: validated; grey triangles: without validation); copy number variations (red, green and yellow lines represent copy number gain, loss or no change (neutral state), respectively); intra-chromosomal translocations (red curve: validated; grey curves: without validation) and inter-chromosomal translocations (green curves).

Supplementary Figure S4. Spectrum of substitutions. Frequency of substitutions for the twelve possible mutations; shown on the y-axis are the various possible nucleotide substitutions; the x-axis represents the frequency of mutations across the genome with that type of mutation.

Supplementary Figure S5. Somatic indel size distribution. A, Distribution of insertion size (1-82bp). B, Distribution of deletion size (1-8,790bp). Since the number of indels > 20 base pairs (bp) is small, we grouped these indels into one bin.

Supplementary Figure S6. FREEC predictions of somatic copy number variations in melanoma with normal sample. Red: gains; Blue: losses; Green: no change.

Supplementary Figure S7. Validation of 128 somatic SNPs by direct sequencing. Seventy putative somatic missense or nonsense SNPs identified by WGS were initially selected for validation by direct sequencing using DNA from the metastatic melanoma and matched blood (Supplementary Table S8). Depth of coverage (DP > 21) and quality (Qual > 37) score cutoff values were established (represented by the shaded areas) so that the SNPs that were not detected in the tumor (red diamonds) in the initial 70 SNPs selected fell below the cutoff. Based on these cutoff scores, we reanalyzed the WGS data and identified an additional 58 somatic nonsynonymous SNPs to validate by direct sequencing. In total, 119/128 (93%) SNPs were detected in the tumor but not in the germline by direct sequencing (blue diamonds).

Supplementary Figure S8. BRAF L597R/Q/S and K601E activate the RAF-MEK-ERK signaling pathway. Immunoblotting of lysates from 293H cells transfected with empty vector (vector) or
plasmids encoding FLAG-BRAF V600E, FLAG-BRAF L597R, FLAG-BRAF L597Q, FLAG-BRAF L597S, and FLAG-BRAF K601E demonstrate that BRAF L597R/Q activate the RAF-MEK-ERK signaling to a lesser degree than BRAF V600E, L597S, and K601E.

**Supplementary Figure S9.** MEK-ERK signaling induced by BRAF L597Q and K601E is sensitive to BRAF and MEK inhibitors. Immunoblotting of lysates from 293H cells transfected with plasmids encoding FLAG-BRAF V600E, FLAG-BRAF L597Q, or FLAG-BRAF K601E demonstrate that MEK-ERK signaling can be inhibited by increasing doses (0, 0.1 μM, 0.5 μM, 1 μm, 5 μM) of **A**, the BRAF inhibitor vemurafenib or **B**, the MEK inhibitor GSK1120212, 2 h post-inhibitor treatment.

**Supplementary Figure S10.** Somatic BRAF L597S mutation confirmation by direct sequencing. Tumor and blood DNA from a 69 year old patient with a metastatic melanoma treated with TAK-733 was extracted and subject to direct sequencing. The arrows indicate the positions of the mutated peaks in the tumor DNA that are absent in the blood DNA. Forward and reverse indicate the use of forward and reverse primers to generate the sequences, respectively.

**Supplementary Figure S11.** Mutation spectrum of melanomas tested in the Vanderbilt clinical lab. From July 1, 2010 to December 31, 2011, 538 melanomas were interrogated by our melanoma SNaPshot assay in Vanderbilt’s CLIA-certified molecular diagnostics laboratory. This assay queries mutations in BRAF (V600), NRAS (G12/13, Q61), KIT (W557, V559, L576, K642, D816), CTNNB1 (S37/45), GNAQ (Q209) and GNA11 (Q209).