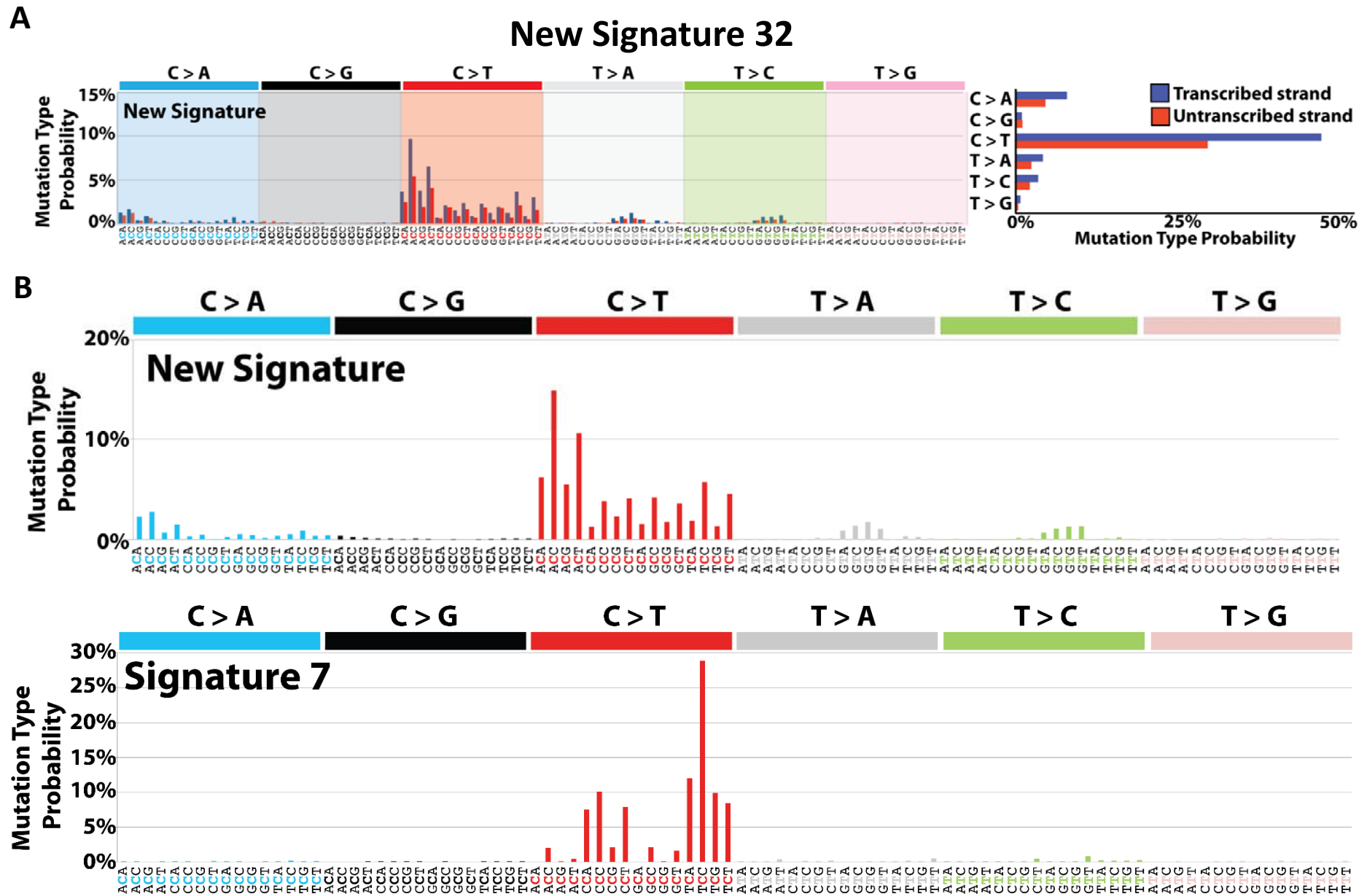


SUPPLEMENTARY INFORMATION

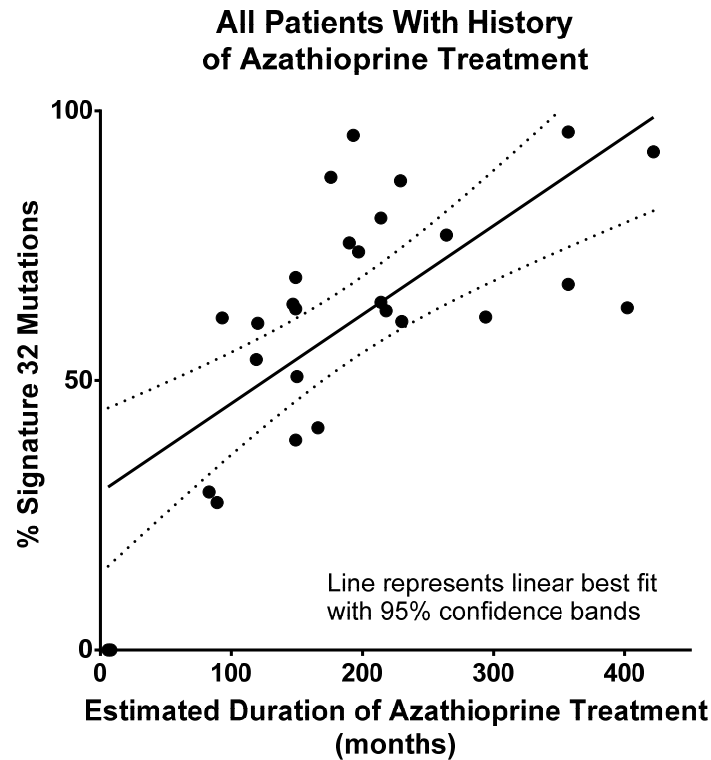
**The genomic landscape of cutaneous SCC reveals drivers
and a novel azathioprine associated mutational signature**

Gareth J Inman et al.



Supplementary Figure 1. Novel signature 32. It is predominately C>T mutations (75%) in combination with C>A, T>A, and T>C mutations. There seems to be a clear presence for C>X at ApCpN and T>X at GpTpN. This novel mutational signature exhibits a very strong transcriptional strand bias potentially indicating an interplay with TC-NER due to adducts on guanine and adenine (A). Comparison between Signature 32 and Signature 7 which is induced by UV exposure (B).

A



C

Correlation			% Signature 32 Mutations
Spearman's rho	Estimated Duration of Azathioprine Treatment (months)	Correlation Coefficient	0.679
		Sig. (2-tailed)**	p < .0001
		N	28

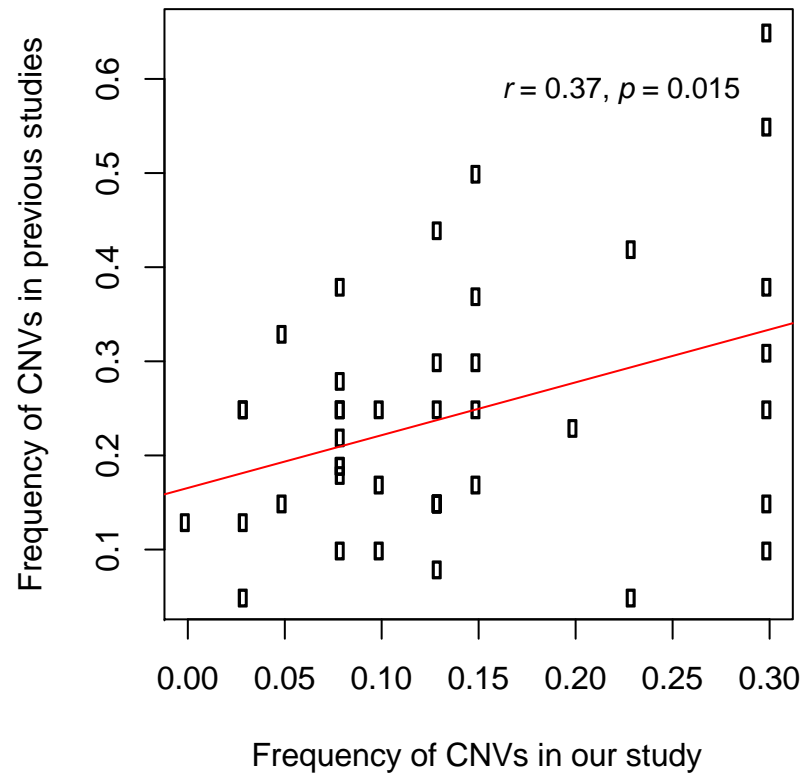
** Correlation is significant at the 0.01 level (2-tailed).

B

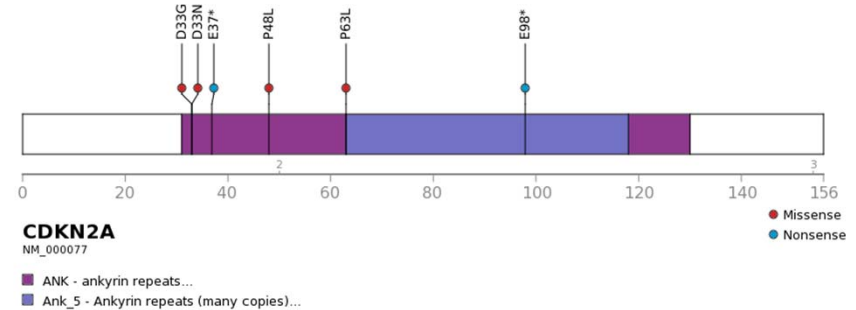
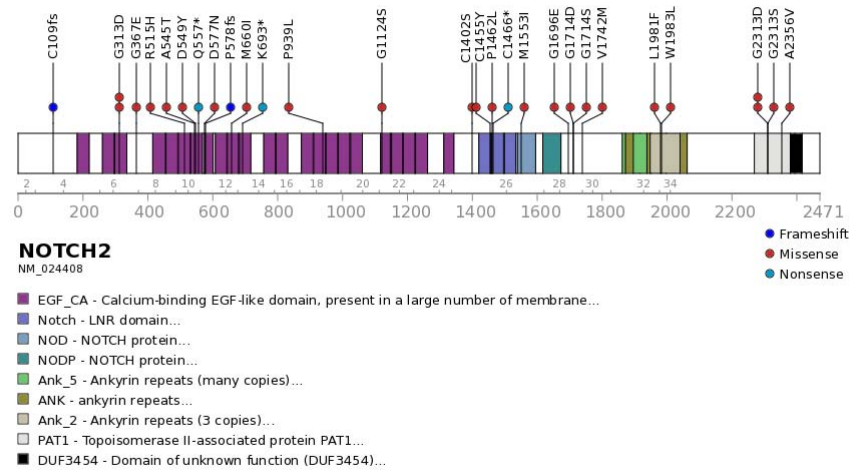
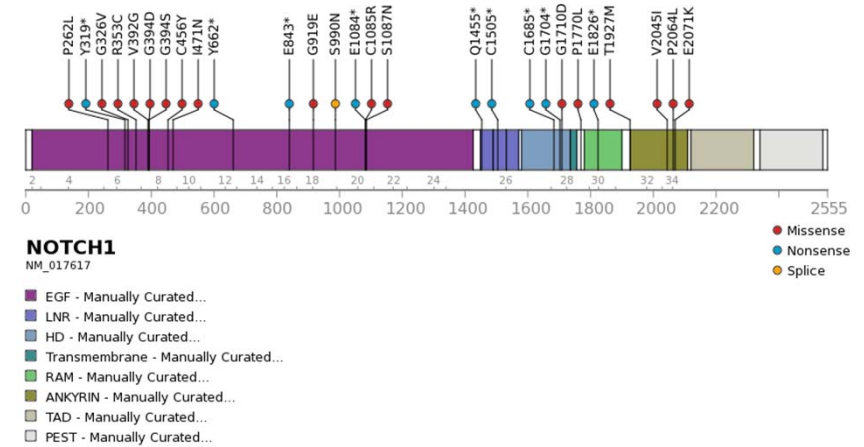
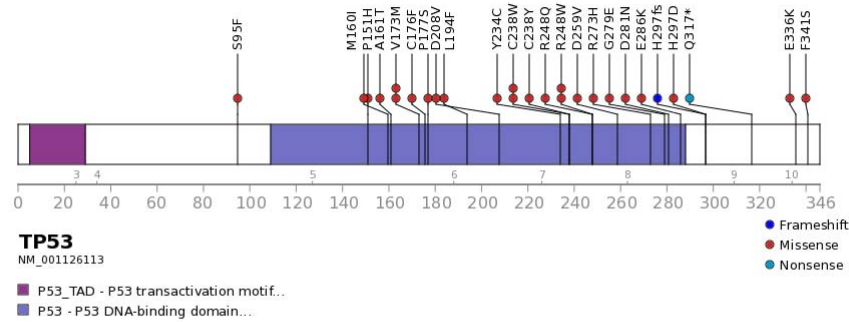
Sample	Estimated Duration of Azathioprine Treatment (months)	% Signature 32 Mutations
MD01	149	38.94%
MD02	150	50.72%
MD04	193	95.49%
MD05	422	92.41%
MD06	402	63.47%
MD16	197	73.87%
PD01	149	63.29%
PD03	149	69.11%
PD04	93	61.62%
PD05	147	64.15%
PD07	294	61.76%
PD08	166	41.22%
PD09	119	53.88%
PD10	6	0.00%
PD11	264	76.98%
WD01	8	0.00%
WD03	89	27.35%
WD05	230	60.95%
WD06	190	75.50%
WD09	120	60.60%
WD11	83	29.32%
WD12	214	80.15%
WD13	357	67.84%
WD14	214	64.52%
WD17	218	62.92%
WD19	357	96.11%
WD20	176	87.71%
WD21	229	87.06%

Supplementary Figure 3. Duration of azathioprine treatment correlates with intensity of signature 32.

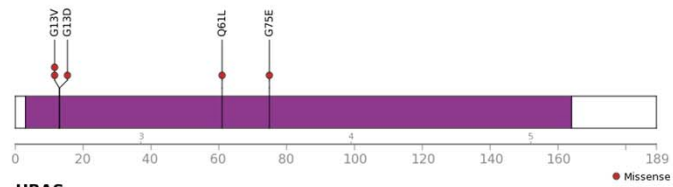
A. Scatterplot of % of signature 32 mutations against estimated duration of azathioprine treatment. B. Table of samples from patients with history of azathioprine use detailing estimate of azathioprine treatment duration (months) and percentage of signature 32 mutations detected in the tumours. C. Spearman's rank-order correlation revealing a statistically significant positive correlation $r_s(26)=0.679$, $p < 0.0001$.



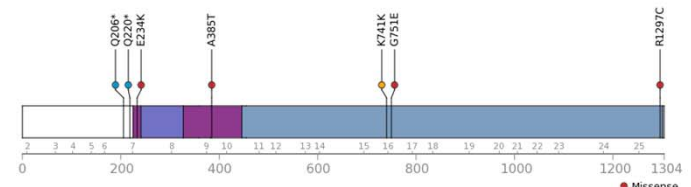
Supplementary Figure 4. Significant positive correlation between this study and previous studies for top frequent CNVs. Previous CNV studies included Salgado et al. Genes, Chromosomes and Cancer 2010, Sekulic et al. Cancer Prev Res 2010, Hameetman et al BMC Cancer 2013 and Purdie et al JID 2009



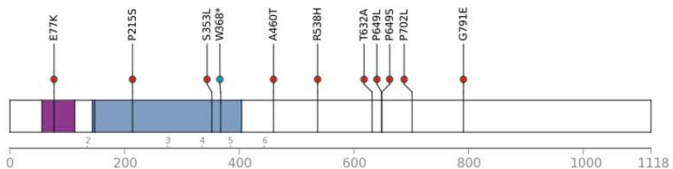
Supplementary Figure 5. Mutational spectrum in 4 SMGs, TP53, NOTCH1, NOTCH2 and CDKN2A



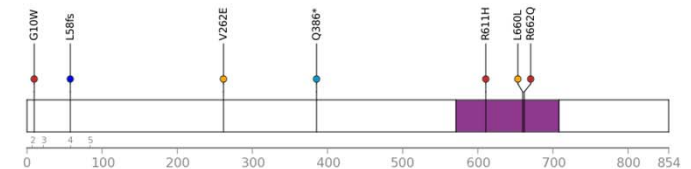
HRAS
 NM_001130442
 H_N_K_Ras_like - Ras GTPase family containing H-Ras,N-Ras and K-Ras4A/4B...



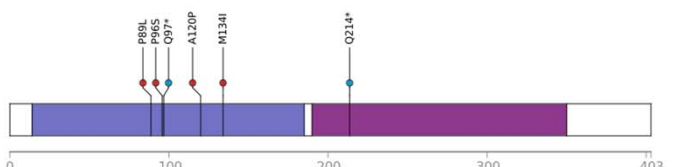
SF3B1
 NM_012433
 Nucleoporin_FG - Nucleoporin FG repeat region...
 CTD - Spt5 C-terminal nonapeptide repeat binding Spt4...
 HSH155 - U2 snRNP spliceosome subunit [RNA processing and modification]...



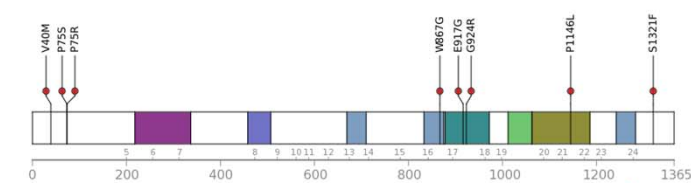
MAP3K9
 NM_033141
 SH3 - Src homology 3 domains...
 TyrKc - Tyrosine kinase, catalytic domain...
 PTKc - Catalytic domain of Protein Tyrosine Kinases...



VPS41
 NM_014396
 CLH - Clathrin heavy chain repeat homology...

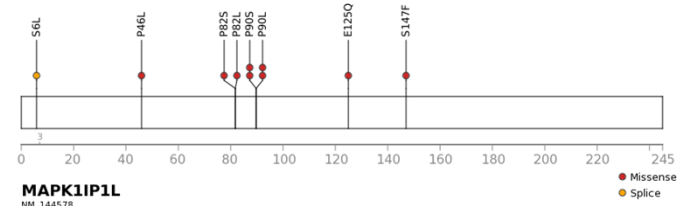
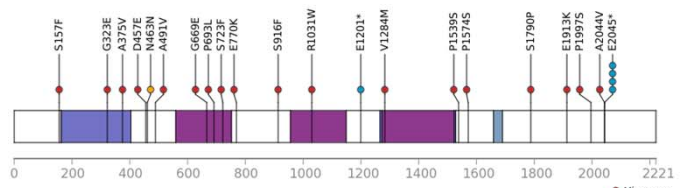
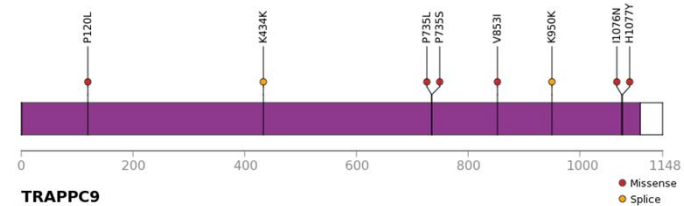
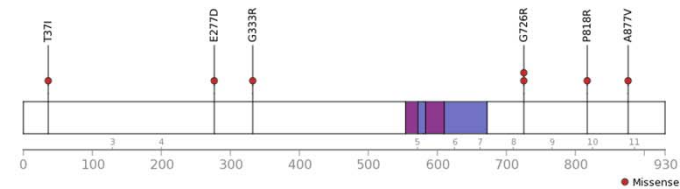
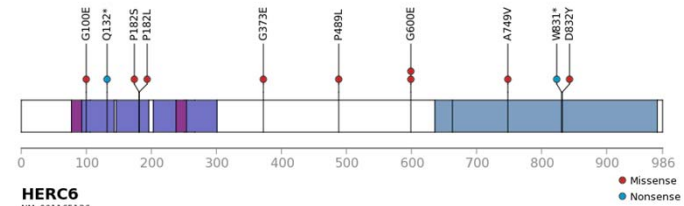
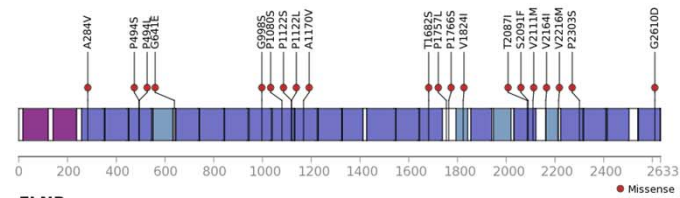


PTEN
 NM_000314
 PTPc - Manually Curated...
 PTEN_C2 - Manually Curated...

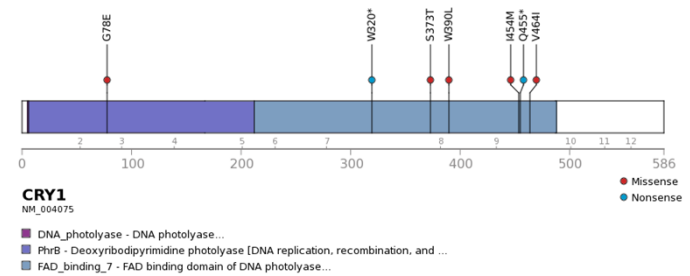
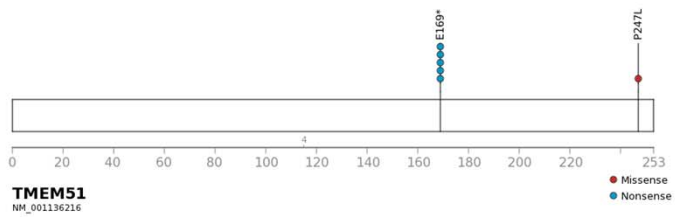
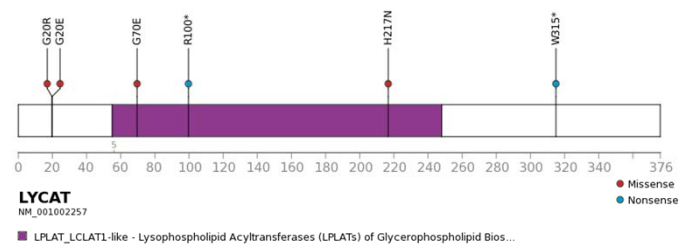
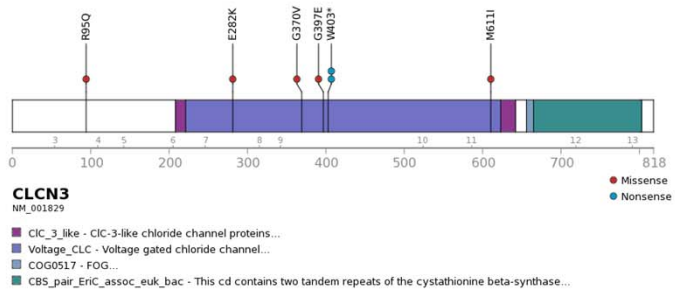
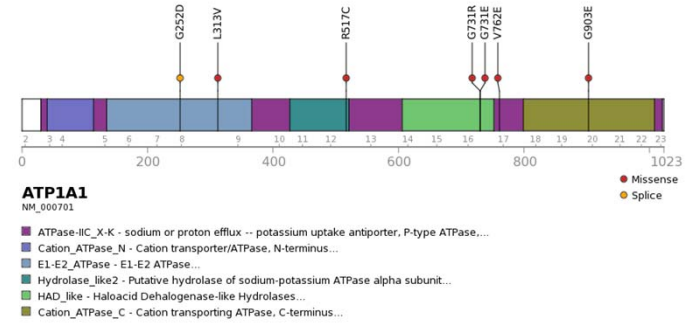
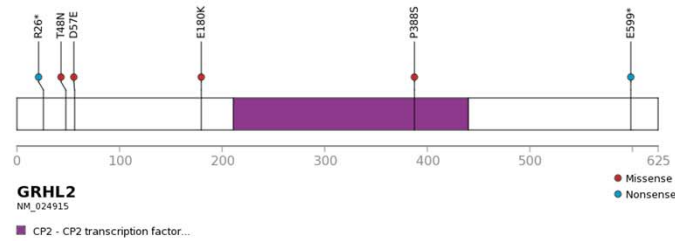


WHSC1
 NM_133330
 MSH6_like - The PWWP domain is present in MSH6, a mismatch repair protein homologo...
 HMG-box - High Mobility Group (HMG)-box is found in a variety of eukaryotic chro...
 RING - RING-finger (Really Interesting New Gene) domain, a specialized type o...
 WHSC1_related - The PWWP domain was first identified in the WHSC1 (Wolf-Hirschhorn syn...
 AWS - associated with SET domains...
 SET - SET domain...

Supplementary Figure 6. Mutational spectrum in HRAS, MAP3K9, PTEN, SF3B1, VPS41 and WHSC1

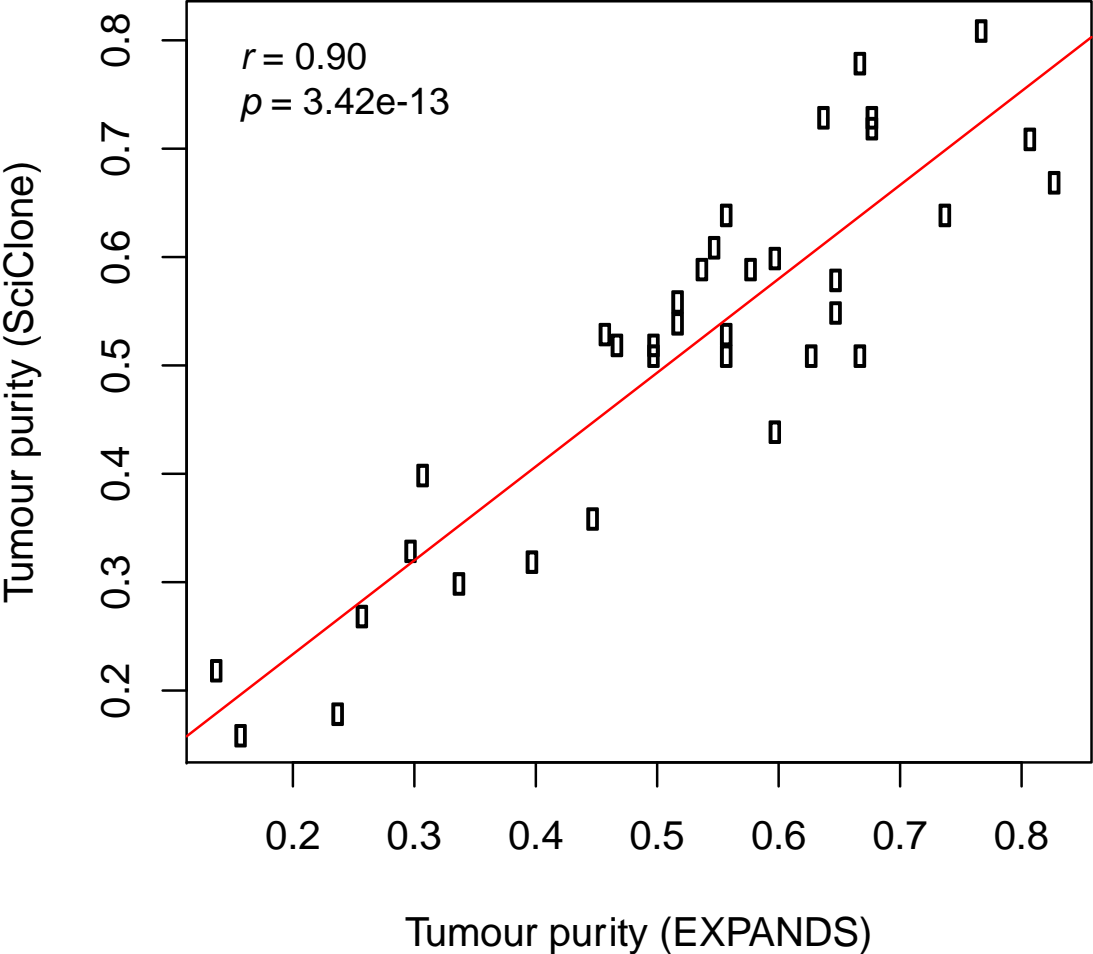


Supplementary Figure 7. Mutational spectrum in FLNB, GLIS3, CACNA1C, HERC6, TRAPPC9 and MAPK1P1L

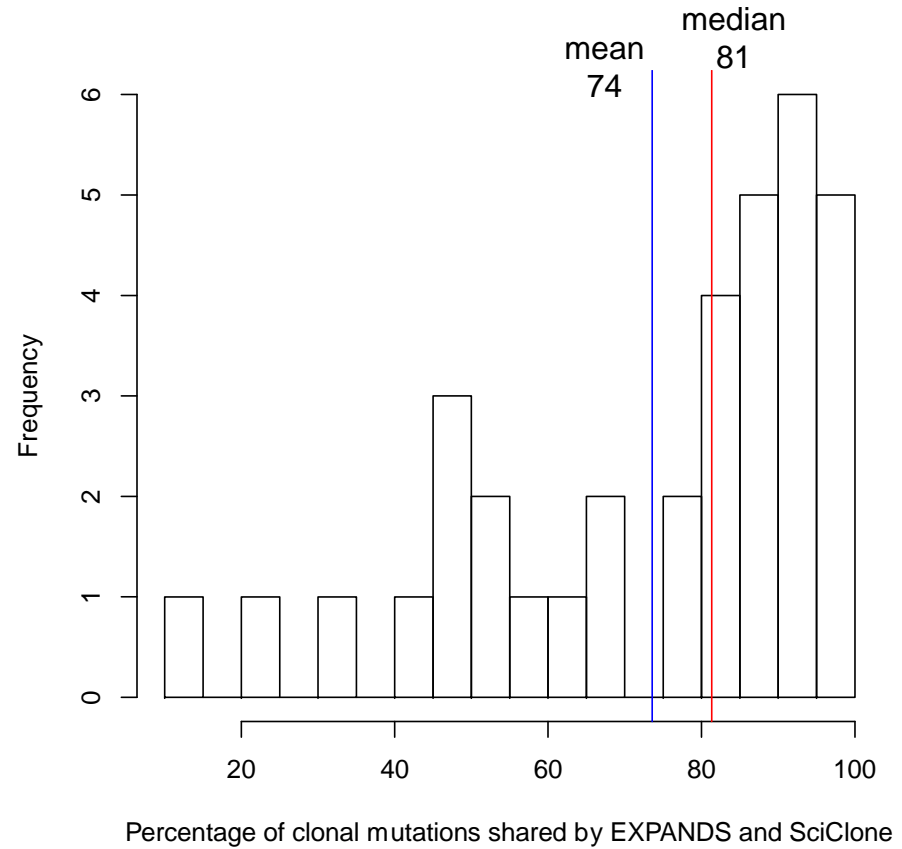
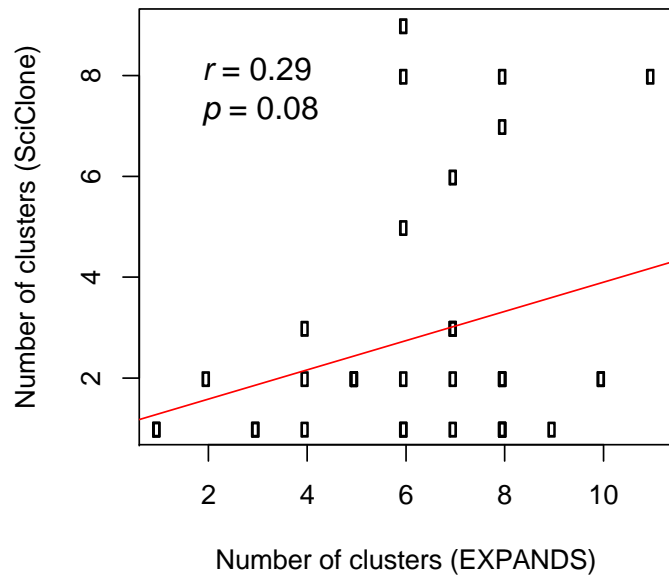


Supplementary Figure 8. Mutational spectrum in GRHL2, CLCN3, TMEM51, ATP1A1, LCLAT1 and CRY1

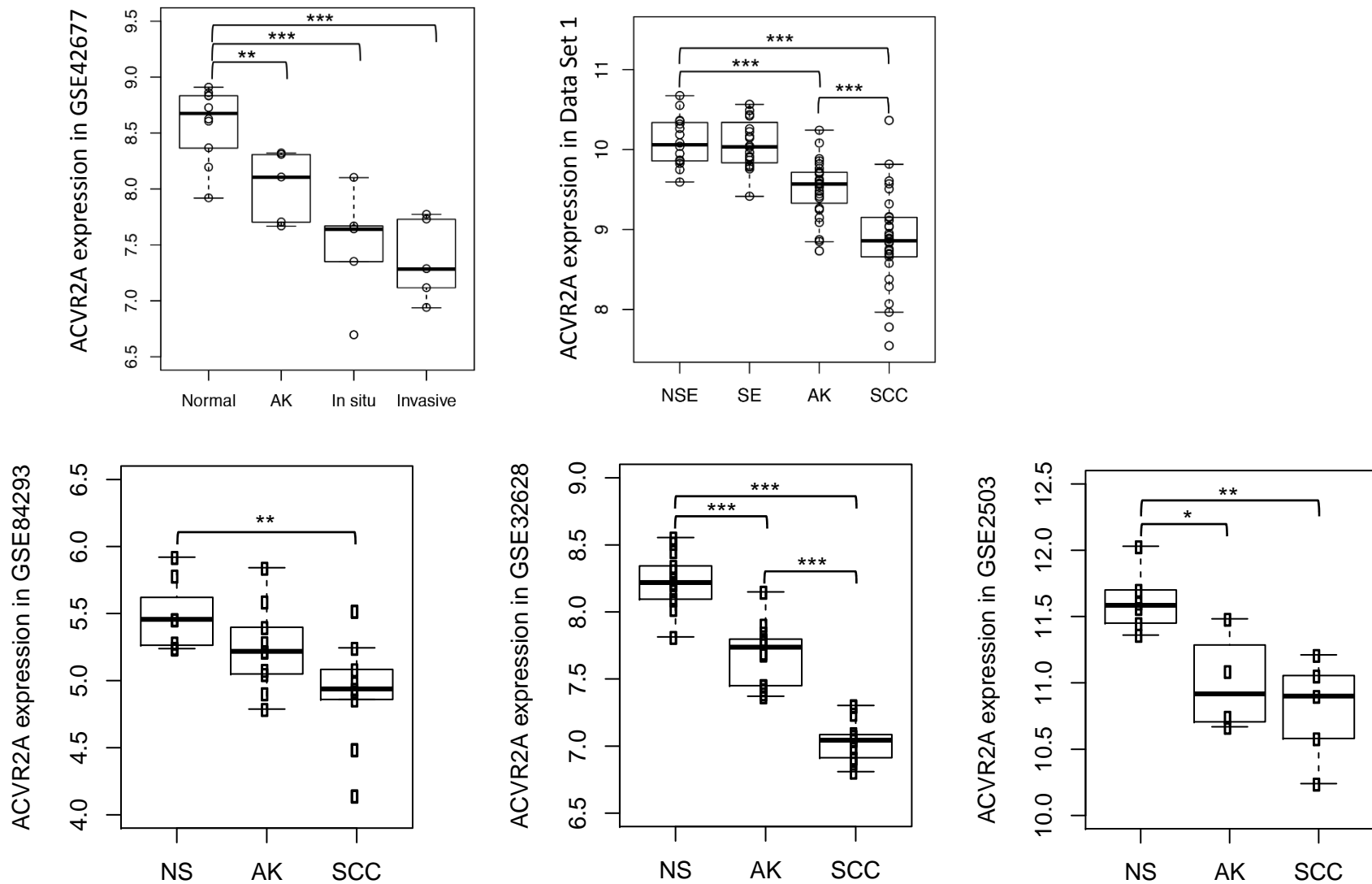
Supplementary Figure 9. Correlation of tumour purity estimated by EXPANDS and SciClone



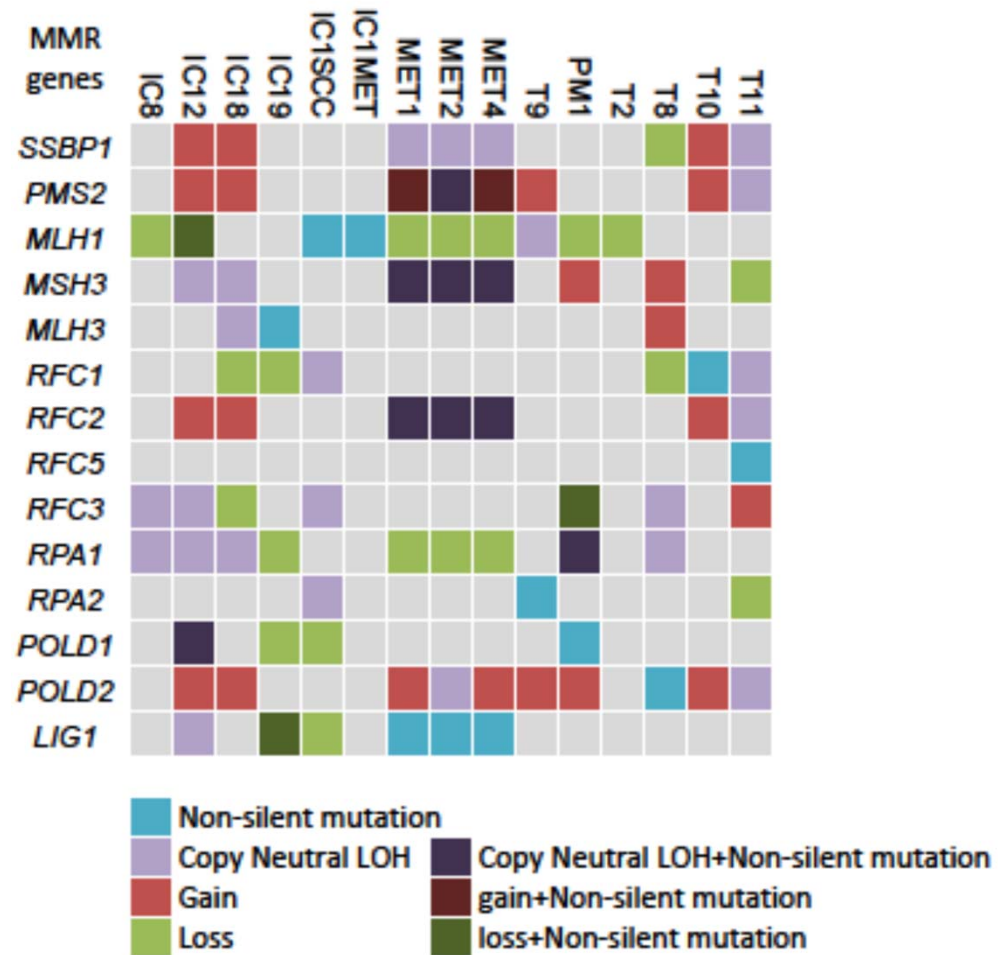
Supplementary Figure 10. Comparison between EXPANDS and SciClone across 35/40 cSCC whole exomes



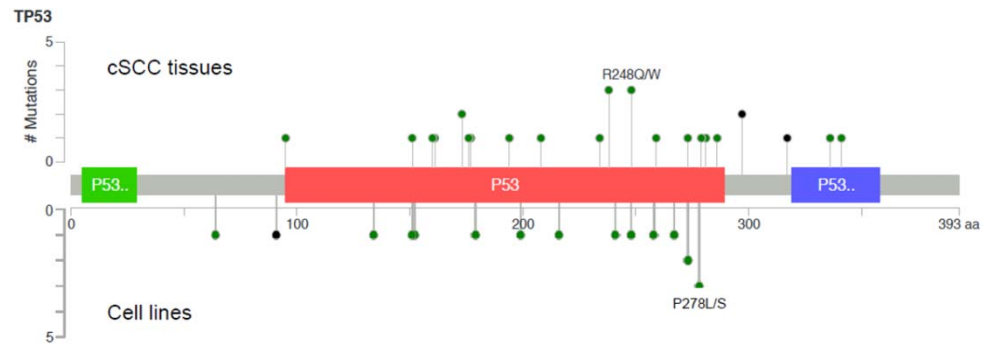
Overall, 74% all 'clonal' mutations called by EXPANDS were also called 'clonal' by SciClone



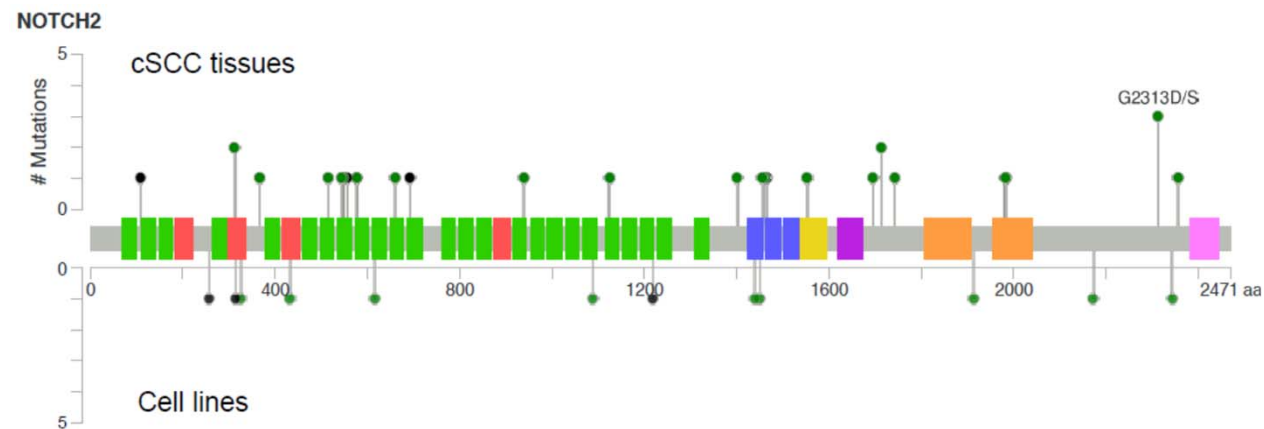
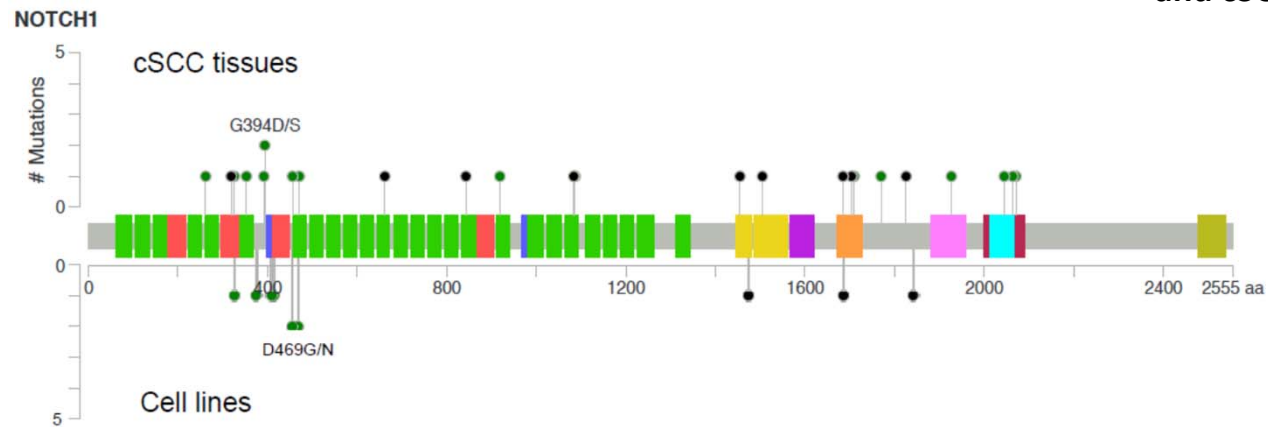
Supplementary Figure 11. ACVR2A expression across NS, AK and SCC groups in 5 gene expression profile (GEP) data sets, GSE42677, GSE45216 plus additional samples (Data set 1), GSE84293, GSE32628 and GSE2503 reveals a significant decrease with disease progression.



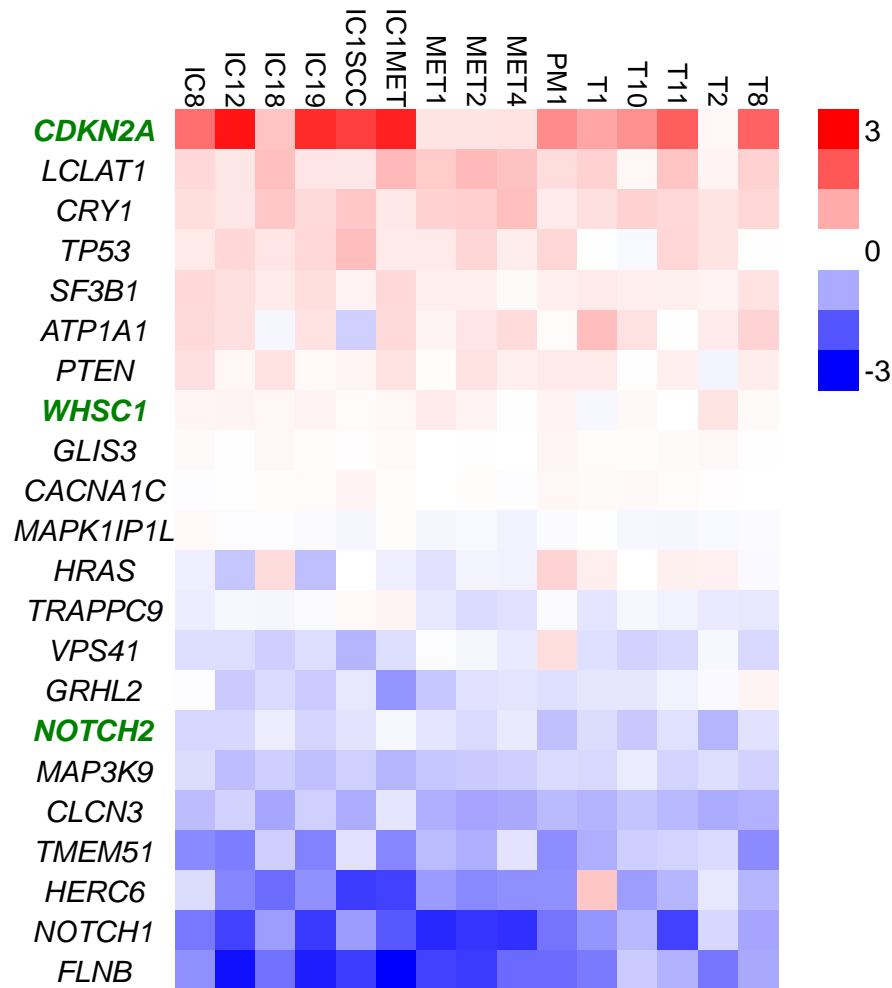
Supplementary Figure 12. OncoPrint of mismatch repair genes in cSCC cell lines



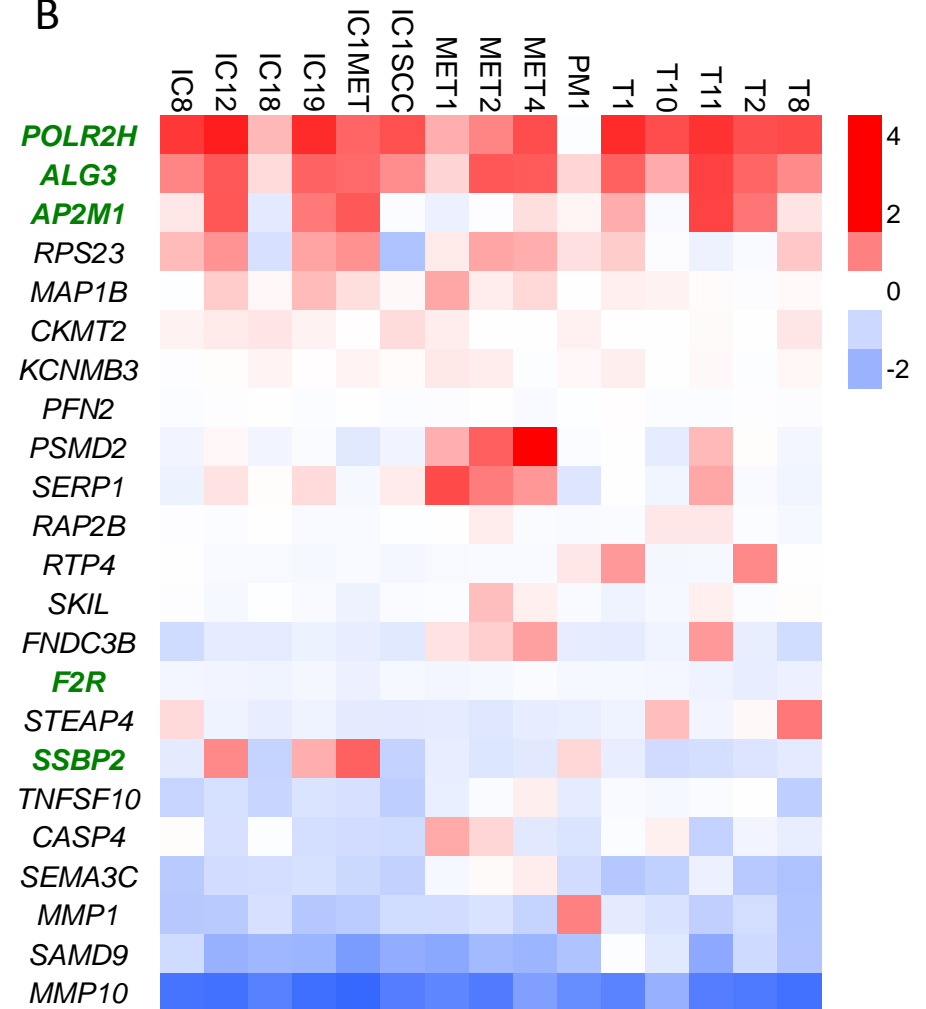
Supplementary Figure 13.
The mutational profiles
of TP53, NOTCH1 and NOTCH2
are similar in primary cSCC tumours
and cSCC cell lines.



A



B



Supplementary Figure 14. Heat map analysis of gene expression changes in cSCC cell lines relative to NHKs.

A) Analysis of the 22 SMGs identified in Figure 3. B) Analysis of the 23 genes of concordant chromosomal alteration identified in Figure 6. One gene NREP had no probe detected. Coincident changes in cell lines and primary tumours are highlighted in green.