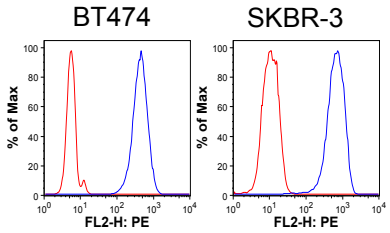
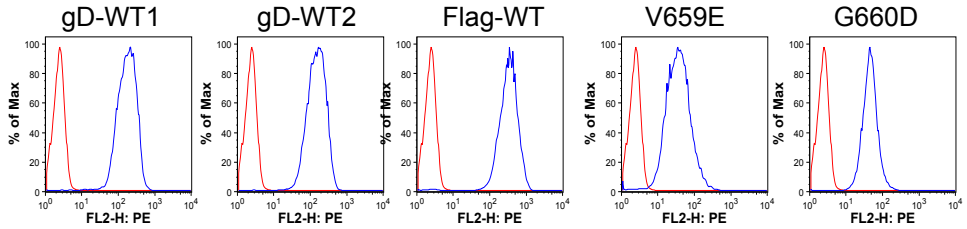
A



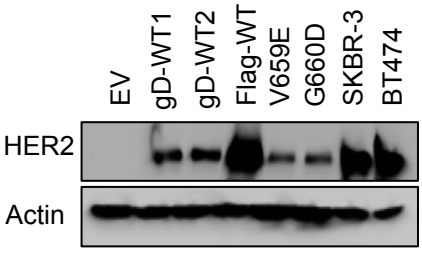
HER2 Copy #	6	10
MFI	469	721

B

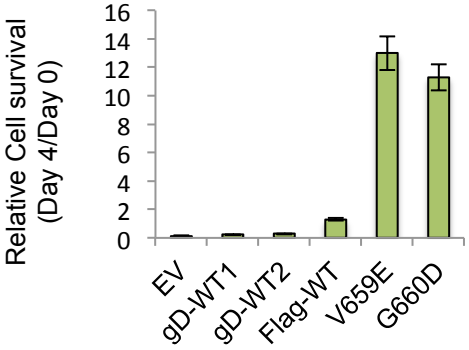


MFI	195	168	371	47	50

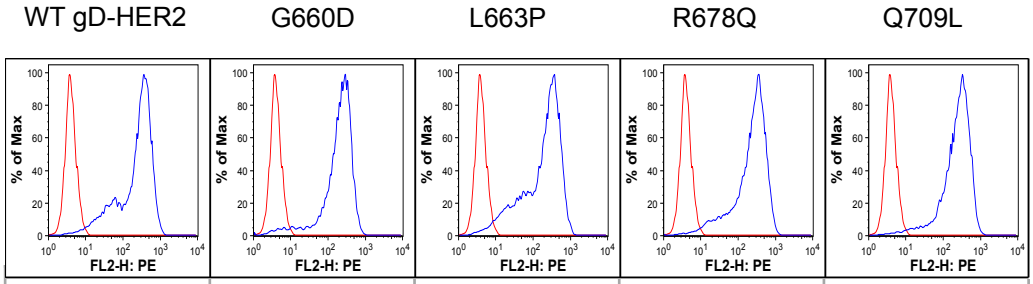
C



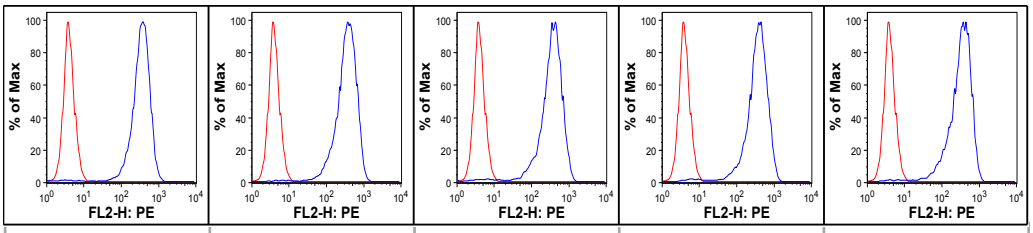
D



E

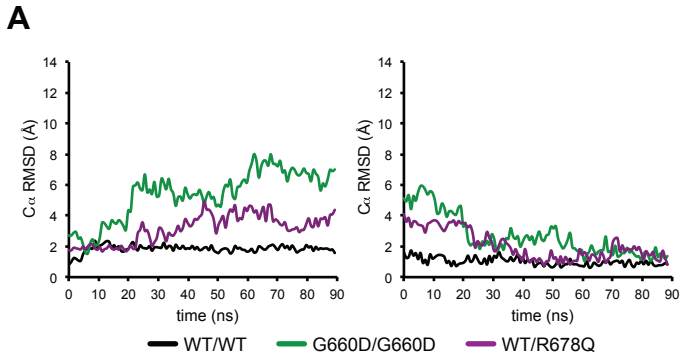


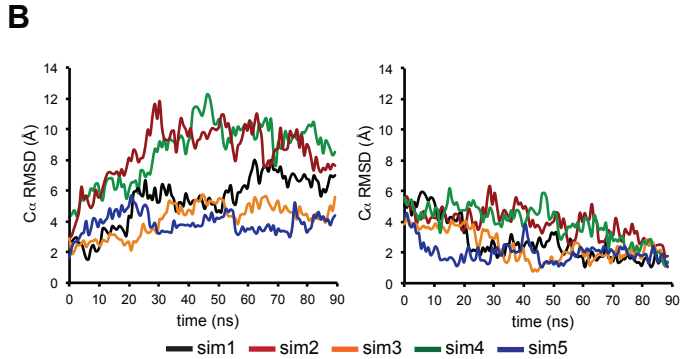
- WT Flag-HER2					
MFI:	317	250	274	310	306



+ WT Flag-HER2					
MFI:	395	415	418	431	383

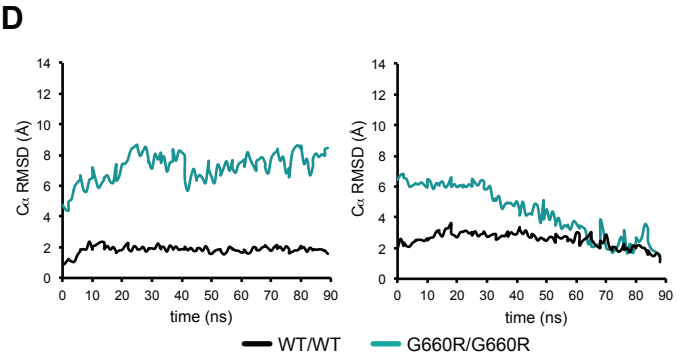
Figure S1, related to Figure 3. HER2 receptor expression analysis. (A) FACS profile of cells incubated with isotype control IgG-PE (red), or anti-HER2-PE antibodies (blue). Mean fluorescence intensity (MFI) provides an estimate of the surface expression of HER2. (B) MFI of BaF3 cells (red), or BaF3 stable cell lines (blue) incubated with anti-HER2-PE antibody measured by FACS. (C) Western blot showing expression of HER2 in BaF3 cells expressing WT or mutant HER2 as indicated. HER2 expression in HER2-amplified breast cancer cell lines is also shown. (D) Relative cell survival of BaF3 cells expressing different levels of WT HER2 or mutant HER2 constructs. Data is presented as mean ± Standard Deviation (SD) of twelve different technical replicates and representative of three independent experiments. (E) Surface expression of HER2 in MCF10A cells stably transformed with empty vector (red histogram, MFI = 4) or HER2 expression constructs (blue histograms) stained with an anti-HER2-PE antibody (BD Biosciences) and read by FACS.





C

sim/sim	sim1	sim2	sim3	sim4	sim5
sim1		4.883	4.320	6.623	4.776
sim2	4.883		6.994	5.240	4.005
sim3	4.320	6.994		7.391	5.569
sim4	6.623	5.240	7.391		5.821
sim5	4.776	4.005	5.569	5.821	



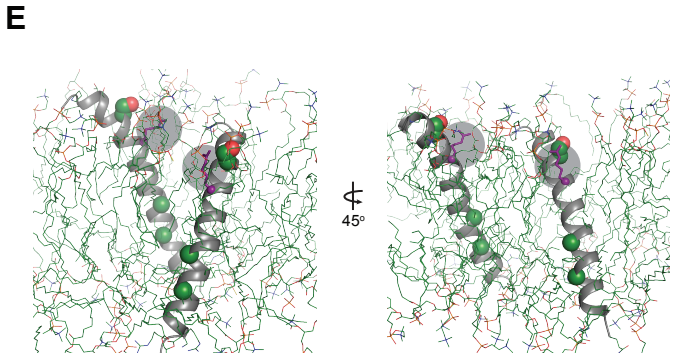


Figure S2, related to Figure 4. **RMSD analysis for MD simulations of wild type and mutant HER2 TMD dimers**. (A) RMSD plot of backbone Cα positions relative to the input model (left panel) and final model (right panel) over the course of the simulation plotted for wild type (WT) HER2 homodimers (WT/WT) (black line), G660D homodimers (green line), and heterodimers of WT HER2 in complex with R678Q (purple line). (B) RMSD analysis for five simulations performed on G660D homodimers to assess the stability of N and C-terminal interfaces in the presence of the G660D mutation. (C) Pairwise RMSD calculation for the final state observed in each independent simulation of HER2 G660D TMD homodimers to assess the degree of convergence between models. (D) RMSD plot for the activating G660R mutant homodimer of HER2 relative to the input model (left panel) and final model (right panel). (E) Final state for MD simulation of a TMD homodimer of HER2 G660R.

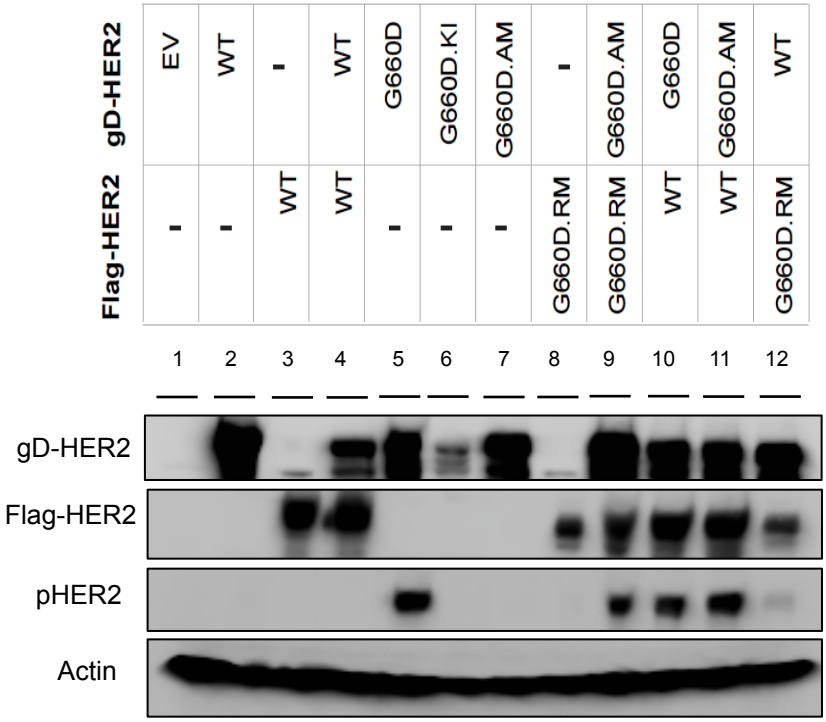


Figure S3, related to Figure 5. Western blot showing expression of HER2, pHER2 in BaF3 cells stably expressing indicated HER2 constructs.

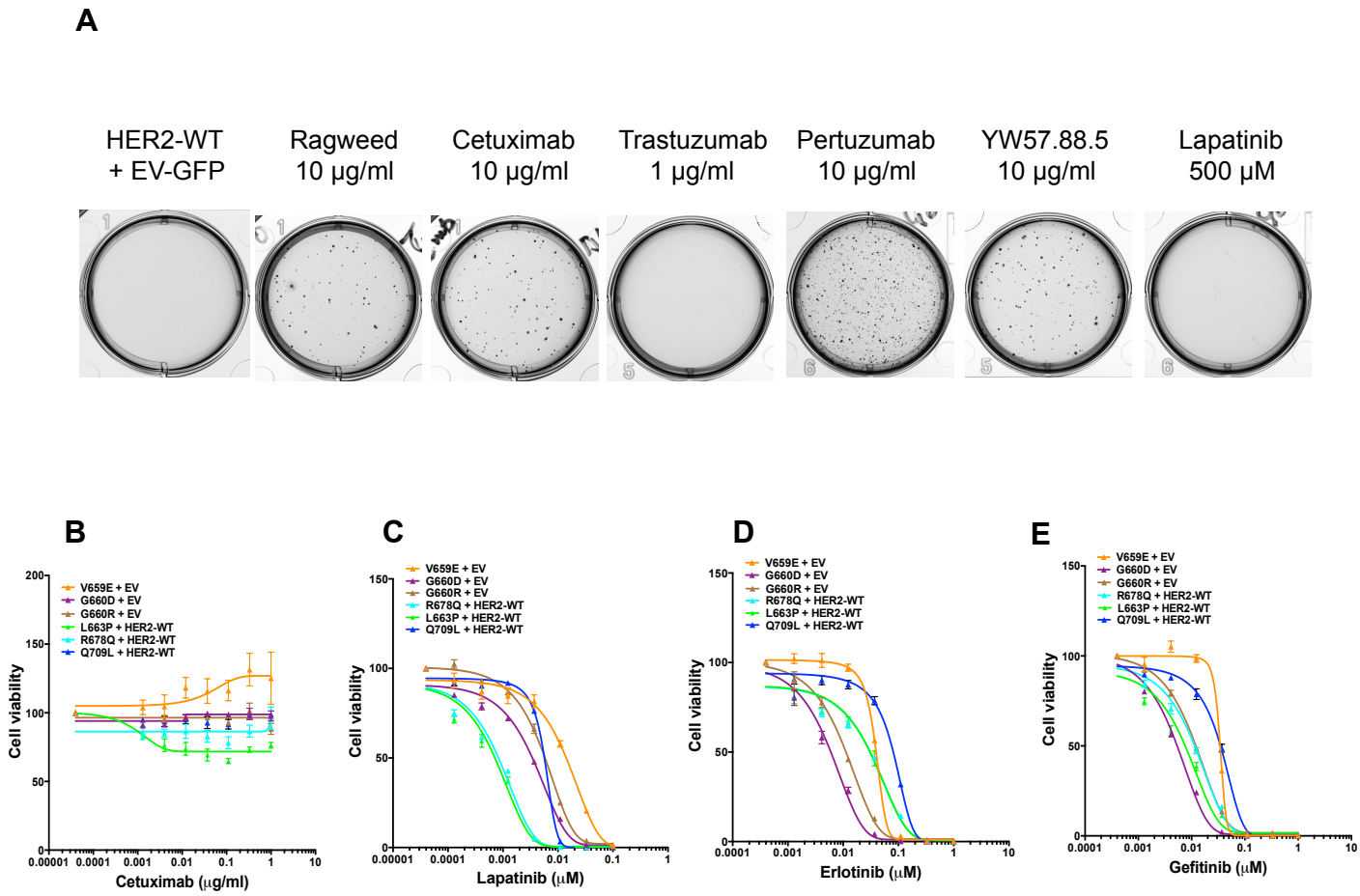


Figure S4, related to Figure 6. (A) Effect of indicated antibody or small molecule drugs on colony formation by BaF3 cells stably expressing HER2 G660D. (B-E) Effect of Cetuximab (B), Lapatinib (C), Erlotinib (D), and Gefitinib (E) on IL-3 independent survival of BaF3 cell expressing the indicated HER2 mutants. Data shown in (B-E) are mean ± Standard error of mean (SEM) of four technical replicates and representative of three independent experiments.

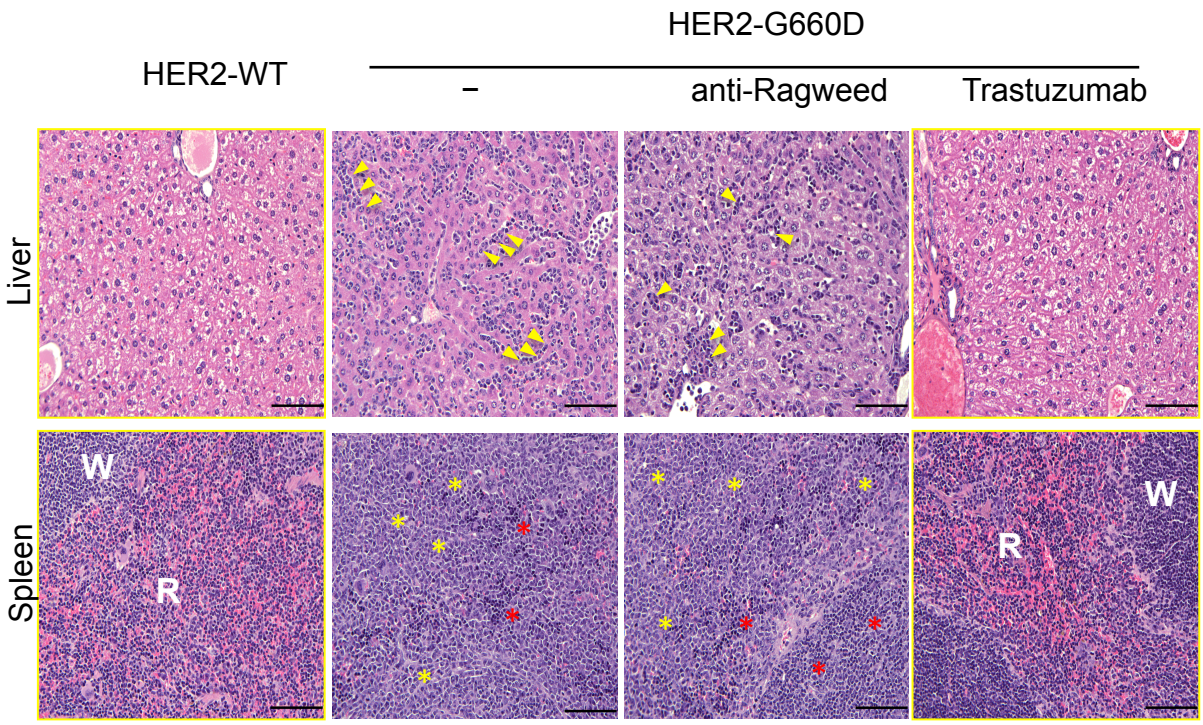


Figure S5, related to Figure 6. **Histological analysis**. H&E staining of liver and spleen sections from mice implanted with BaF3 cells stably expressing HER2-WT or HER2-G660D treated with either anti-Ragweed antibody or trastuzumab (see Figure 6G). Infiltrating tumor cells in sinusoids in liver (yellow arrows) and spleen (yellow asterisk) are shown. Normal lymphocytes are indicated by red asterisk. R, red pulp; W, lymphoid follicles of white pulp. Scale bars shown at the bottom of each image represent 100μm.

Table S3, related to Figure 6 - IC_{50} of Trastuzumab and Pertuzumab against HER2 TMD/JMD mutants

IC_{50} of antibodies treament on BaF3-cells expressing HER2-mutants		
HER2 mutant	IC_{50} (ng/ml)	
	Trastuzumab	Pertuzumab
V659E + EV	1.515	ND
G660D + EV	0.8606	ND
G660R + EV	2.763	ND
L663P + WT	0.5241	2.096
R678Q + WT	1.664	3.618
Q709L + WT	7.045	16.68

ND = Not determined - stimulatory or not effective; EV = empty vector; WT = wildtype HER2

Table S4, related to Figure 6 - IC_{50} of Kinase small molecule inhibitors tested against HER2 TMD/JMD mutants

HER2 mutant	IC_{50} (nM)				
	Neratinib	Afatinib	Erlotinib	Gefitinib	Lapatinib
V659E + EV	0.5874	17.97	39.99	32.45	17.97
G660D + EV	0.252	1.657	4.912	4.54	3.849
G660R + EV	0.4474	2.879	10.85	11.54	5.662
L663P + WT	0.1235	0.3415	35.03	4.685	0.4969
R678Q + WT	0.1274	0.2568	34.34	10.39	0.6688
Q709L + WT	0.3302	1.454	83.29	37.42	5.206

EV = empty vector; WT = wildtype HER2

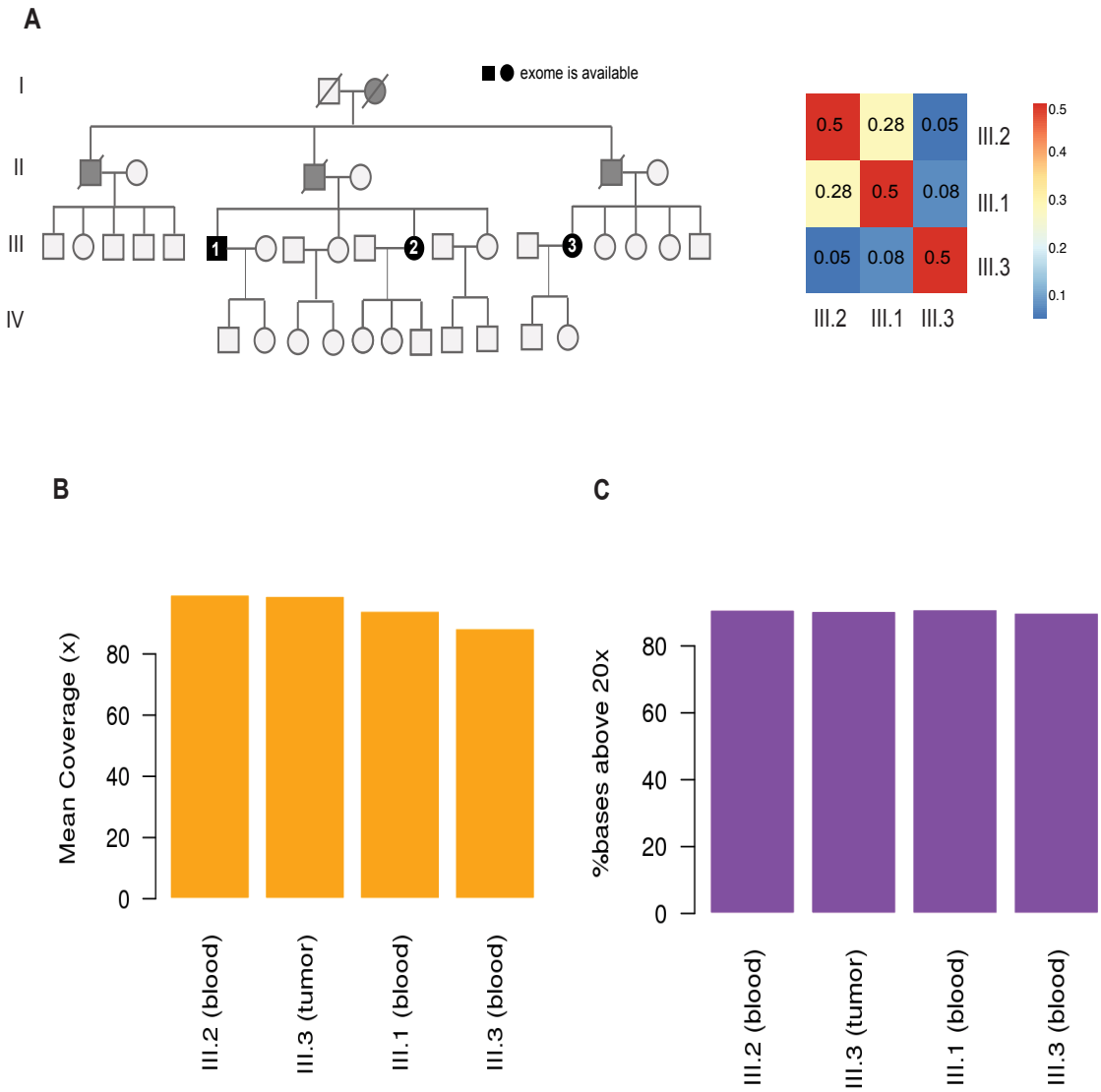


Figure S6, related to Figure 7. Exome analysis. (A) Pedigree chart and kinship coefficients showing the expected relatedness of the three affected individuals. (B) Sequencing coverage summary. (C) Percent bases sequenced at least 20x.

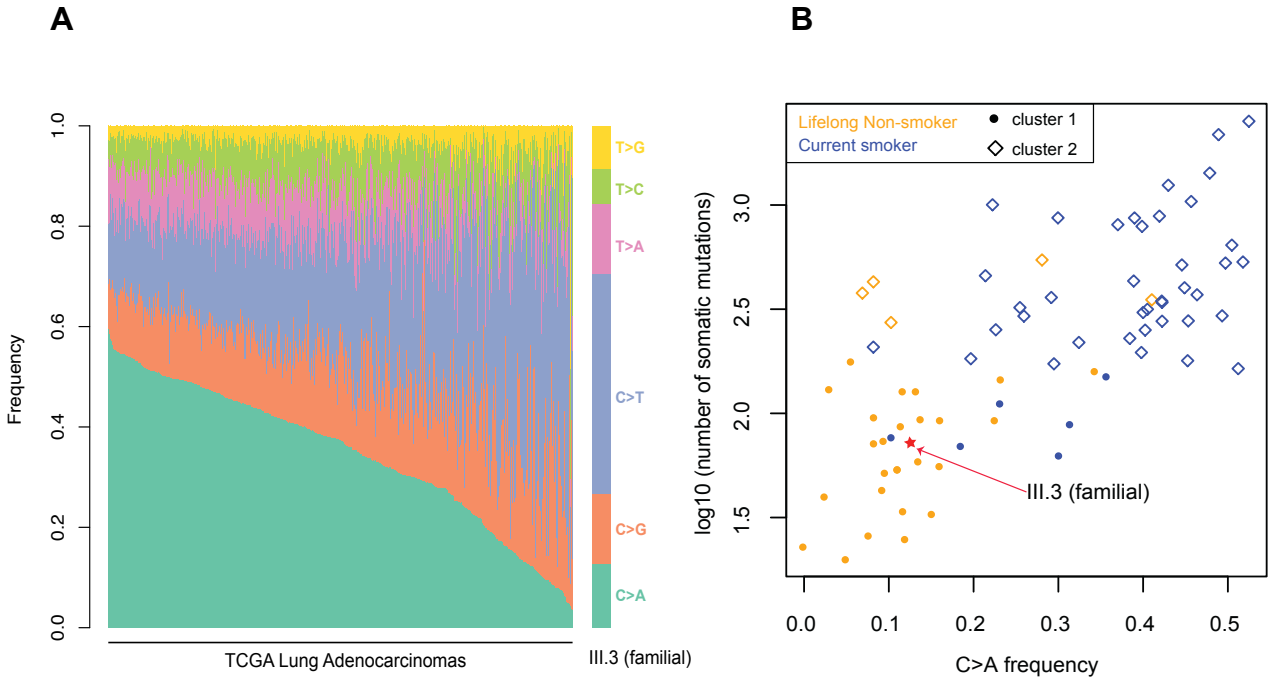


Figure S7, related to Figure 7. (A) Frequency of the six possible base substitutions in lung tumors samples from the cancer genome atlas (TCGA) analysis compared to the tumor sample from the familial lung cancer patient III.3. (B) C>A substitution in smokers vs non-smokers.