SUPPLEMENTAL METHODS

***In silico* data set**

The Multi-Center Mutation calling in Multiple Cancers (MC3) data were generated using seven different mutation calling algorithms from four centers across 32 different tumor types, thus representing a uniform set of mutation calls across thousands of tumor/normal pairs. The authors reported they focused their analysis on independently determined consensus exome coding regions defined by CCDS (GRCh37.p13, GCF_000001405.25) that were likely captured for each sample.[1]
We extracted the variants in the mc3.v0.2.8.PUBLIC.maf file (https://gdc.cancer.gov/about-data/publications/pancanatlas) that overlapped with the CCDS, using bedtools[2] (https://bedtools.readthedocs.io/en/latest/content/bedtools-suite.html). The bed file was intersected (using bedtools -wa option) with the mc3.maf file. Finally, the data was filtered for any overlap or redundancy using the 'merge' function. The Consortium created a final bed file that covered 32.102Mb of the genome, which served as the denominator for calculating WES.TMB. Three different Consortium laboratories independently calculated TMB using the same dataset and analytical methodology with 100% concordance.

Statistical analysis

Statistical analyses began with descriptive numerical and graphical summaries of the data to interrogate the relationship between WES.TMB and panel.TMB values. First analyses focused on the combined data from all 32 tumor types. For each panel, a scatterplot was constructed with WES.TMB on the x-axis and panel.TMB on the y-axis, and Spearman R was calculated. Scatterplots and difference plots (panel.TMB minus WES.TMB on y-axis and WES.TMB on x-axis) were examined to assess linearity of the relationship between panel.TMB and WES.TMB and to evaluate whether variance of panel.TMB was constant across the range of WES.TMB values. These assessments informed modeling of the relationships between panel.TMB and WES.TMB.

Descriptive analyses suggested that the 32 tumors could be divided roughly into three strata. Stratum 1 contained 8 tumors (Supplemental Table 4A-Stratum 1) that demonstrated TMB values spanning the full range 0-40. Stratum 2 tumors (Supplemental Table 4B-Stratum 2) had mostly low TMB values but an occasional TMB value in the upper end of the range. Stratum 3 tumors (Supplemental Table 4C-Stratum 3) had TMB values concentrated at the low end of the range. These characteristics suggested that regression models for stratum 2 tumors would likely be less stable than those for stratum 1 as they would be highly influenced by the occasional tumors with TMB values in the upper end of the range. Regression modeling was judged to be futile for the stratum 3 tumors which spanned such a narrow range of TMB values relative to variability in panel.TMB values, for a given value of WES.TMB, that any regression model would be highly unreliable. Consequently, it was judged that regression modeling for individual tumor types would focus on tumor types in stratum 1.

Regression modeling was conducted separately for each panel. First, a regression model was fit for the combined set of 32 tumors, and then models were fit separately by the three strata. Separate regression models were fit for individual tumor types in stratum 1, but tumor type-specific regression fits were considered unreliable for strata 2 and 3 for reasons discussed above. Since the variability in panel.TMB values about the fitted regression line was observed to increase with the mean (and with WES.TMB), the regression models allowed for heteroscedasticity in the errors but assumed independence between observations. Weighted least squares was used for the regression modeling to account for the observed heteroscedasticity in errors, i.e., the variability in panel.TMB values about the fitted regression line was observed to increase with the mean (and with WES.TMB). For each regression,

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the mean panel.TMB was modeled as a simple linear function of the WES.TMB, and five different models for the error variance were considered. Restricted maximum likelihood (REML) analysis using the gls function available in the R package nlme[3,4] was performed to estimate the model parameters and select a best fitting variance structure based on minimum AIC and BIC criteria.

The five different variance models that were initially considered in the REML analyses each assumed that residual errors followed a Gaussian distribution, but with different variance functions as follows:

1. Constant (homoscedastic) function: $var(panel.TMB|WES.TMB) = \sigma^2$ where σ^2 is the variance parameter that is estimated using R allowing the default equal weights option in the gls function call (equivalent to ordinary least squares regression).
2. Power function: $var(panel.TMB|WES.TMB) = \sigma^2|WES.TMB|^{2c}$ where 2*c* defines the power in the variance function and σ^2 is a scale factor. These variance parameters are estimated using R, specifying

   weights = varPower(form=~WES.TMB)

   in the gls function call.
3. Exponential function: $var(panel.TMB|WES.TMB) = \sigma^2\exp(2c * WES.TMB)$ where 2*c* defines the rate parameter in the exponential variance function and σ^2 is a scale factor. These variance parameters are estimated using R, specifying

   weights = varExp(form=~WES.TMB)

   in the gls function call.
4. Constant plus power function: $var(panel.TMB|WES.TMB) = (c_1 + |WES.TMB|^{c_2})^2$ where c_1 and c_2 define the location and scale parameters of the variance function that are estimated using R, specifying

   weights = varConstPower(0.5,0.5,form=~WES.TMB)

   in the gls function call (initializing each of the constants c_1 and c_2 to 0.5).
5. Cancer type-specific power function: Same as variance model 2 except that the power parameter, *c*, is allowed to depend on the cancer type. These variance parameters are estimated using R, specifying

   weights = varPower(form=~WES.TMB|Cancer.type)

   in the gls function call.

A linear form of the mean structure was found to provide a reasonable fit for each of the panels. The power variance model was found to provide the best fit or near-best fit variance structure (by minimum AIC and BIC criteria) across all regression analyses. A final model comprising a linear mean structure and power variance structure was fit by maximum likelihood for each regression. Using parameters estimated from these final models, 95% prediction intervals were constructed, as a function of WES.TMB value, to provide approximate bounds within which 95% of the panel.TMB values are expected to fall. The approximate 95% prediction limits (LL, UL) for the distribution of panel.TMB values expected when WES.TMB=w_0 are calculated as

$$LL = \hat{b}_0 + \hat{b}_1 w_0 - q_t(0.975, n-2)\hat{\sigma} w_0^{\hat{c}}(1 + 1/n + (w_0 - \overline{w})^2/[(n-1)s_w^2])^{\frac{1}{2}}$$

$$UL = \hat{b}_0 + \hat{b}_1 w_0 + q_t(0.975, n-2)\hat{\sigma} w_0^{\hat{c}}(1 + 1/n + (w_0 - \overline{w})^2/[(n-1)s_w^2]))^{\frac{1}{2}}$$

where

$w_1, w_2, \ldots, w_n$ are the *n* observed WES.TMB values;

$\overline{w} = \sum_{i=1}^{n} w_i/n$;

$s_w^2 = \sum_{i=1}^{n}(w_i - \overline{w})^2/(n-1)$;

$\hat{b}_0$ is the estimated intercept of the regression line fit by gls;

$\hat{b}_1$ is the estimated slope of the regression line fit by gls;

$\hat{c}$ and $\hat{\sigma}$ are the estimated parameters of the power variance function as defined in 2 above;

$q_t(0.975, n-2)$ is the 97.5th percentile (0.025 upper quantile) of a t-distribution with *n*–2 degrees of freedom. For 99% prediction limits, $q_t(0.995, n-2)$ replaces $q_t(0.975, n-2)$.

References:

1. Ellrott, K. *et al.* Scalable Open Science Approach for Mutation Calling of Tumor Exomes Using Multiple Genomic Pipelines. *Cell Syst.* **6**, 271-281.e7 (2018).

2. Quinlan, A. R. & Hall, I. M. BEDTools: A flexible suite of utilities for comparing genomic features. *Bioinformatics* **26**, 841–842 (2010).

3. R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. R package version 3.5.1, < URL https://www.R-project.org/>.

4. Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2018). _nlme: Linear and Nonlinear Mixed Effects Models_. R package version 3.1-137, <URL: https://CRAN.R-project.org/package=nlme>.

SUPPLEMENTAL TABLES

Supplementary Table 1. Tumor counts by stratum and histology

A. Stratum 1: 8 Tumor Histologies Covering Full WES TMB Range

Histology	**Total number of cases**	**Number of cases with WES TMB ≤ 5**	**Number of cases with WES TMB between 5-10 mut/Mb**	**Number of cases with WES TMB between 10-40 mut/Mb**	**Number of cases with WES TMB ≥ 40 mut/Mb**
BLCA	195	105	57	33	0
COAD	143	108	5	15	15
HNSC	233	193	25	14	1
LUAD	229	106	62	60	1
LUSC	228	86	110	32	0
SKCM	182	53	45	68	16
STAD	195	129	22	38	6
UCEC	222	134	17	46	25
Total	**1627**	**914**	**343**	**306**	**64**

Abbreviations: Mb, megabase; mut, mutation; TMB, tumor mutational burden; WES, whole exome sequencing

B. Stratum 2: 11 Tumor Histologies Covering Limited WES TMB Range

Histology	**Total number of cases**	**Number of cases with WES TMB ≤ 5**	**Number of cases with WES TMB between 5-10 mut/Mb**	**Number of cases with WES TMB between 10-40 mut/Mb**	**Number of cases with WES TMB ≥ 40 mut/Mb**
ACC	44	40	2	2	0
BRCA	367	350	9	7	1
CESC	143	119	16	7	1
ESCA	90	80	8	1	1
GBM	151	149	0	1	1
KIRC	179	178	0	1	0
LGG	242	241	0	1	0
LIHC	177	169	6	2	0
PRAD	205	203	1	1	0
READ	44	41	1	1	1
SARC	112	108	2	2	0
Total	**1754**	**1678**	**45**	**26**	**5**

Abbreviations: Mb, megabase; mut, mutation; TMB, tumor mutational burden; WES, whole exome sequencing

C. Stratum 3: 13 Tumor Histologies Covering Low WES TMB Range Only

Histology	**Total number of cases**	**Number of cases with WES TMB ≤ 5**	**Number of cases with WES TMB between 5-10 mut/Mb**	**Number of cases with WES TMB between 10-40 mut/Mb**	**Number of cases with WES TMB ≥ 40 mut/Mb**
CHOL	18	18	0	0	0
DLBC	16	14	2	0	0
KICH	27	27	0	0	0
KIRP	126	126	0	0	0
MESO	38	38	0	0	0
OV	31	30	1	0	0
PAAD	58	58	0	0	0
PCPG	75	75	0	0	0
TGCT	64	64	0	0	0
THCA	190	190	0	0	0
THYM	42	42	0	0	0
UCS	28	27	1	0	0
UVM	40	40	0	0	0
Total	**753**	**749**	**4**	**0**	**0**

Abbreviations: Mb, megabase; mut, mutation; TMB, tumor mutational burden; WES, whole exome sequencing

Supplemental Table 2: Parameters for the Uniform TMB Calculation Method used in Phase 1 of the *Friends* TMB Harmonization Project- the *In silico* Analysis

Parameter	Uniform method
Types of mutations counted	Missense_Mutation In_Frame_Del Nonsense_Mutation In_Frame_Ins Frame_Shift_Del Frame_Shift_Ins
Sample QC metric	if ≥50% of variants from a sample contain variants which did not pass quality filter, sample is removed from analysis.
Coverage	Median coverage >300X
Variants excluded	"NOT PASS"
Variant allele frequency (VAF)	≥ 0.05
Tumor_part_depth (t_depth)- coverage*	≥25
Minimum variant count (t_alt_count)*	≥3
Equation for determination of denominator	Stop minus start
Denominator	32.102474 Mb
Capping of TMB values for sensitivity and specificity	40 Mut/Mb

Abbreviations: Del, deletions; Ins, insertions; Mb, megabase; mut, mutation; QC, quality control; TMB, tumor mutational burden

Supplemental Table 3: Intercept and Slope Estimates for All Participating Laboratories, by Stratum

Lab	Intercept	95% CI	Slope	95% CI	Δ	Spearman R
Stratum 1						
1	1.36	1.23-1.49	1.07	1.05-1.10	0.05	0.86
2	-0.03	-0.21	1.08	1.05-1.10	0.05	0.9
3	0.63	0.51-0.75	1.04	1.01-1.06	0.05	0.86
4	0.23	0.16-0.30	0.9	0.88-0.92	0.04	0.9
5	0.63	0.51-0.76	1.07	1.04-1.09	0.05	0.88
6	1.04	0.93-1.15	0.8	0.78-0.82	0.04	0.84
7	0.16	0.06-0.25	0.99	0.96-1.01	0.05	0.9
8	1.92	1.72-2.12	1.32	1.28-1.36	0.08	0.82
9	1.9	1.73-2.06	1.1	1.06-1.13	0.07	0.81
10	0.38	0.26-0.50	0.97	0.95-1.00	0.05	0.85
11	0.77	0.65-0.88	0.9	0.87-0.93	0.06	0.86

Stratum 2						
1	0.83	0.75 − 0.91	1.17	1.12 − 1.21	0.09	0.61
2	−0.17	−0.23 − −0.12	1.06	1.01 − 1.11	0.1	0.66
3	0.26	0.20 − 0.32	1.02	0.97 − 1.07	0.1	0.6
4	0.24	0.21 − 0.28	0.84	0.81 − 0.87	0.06	0.71
5	0.33	0.26 − 0.40	1.1	1.05 − 1.15	0.1	0.64
6	0.51	0.44 − 0.58	0.9	0.85 − 0.94	0.09	0.57
7	−0.13	−0.19 − −0.08	1.07	1.03 − 1.11	0.08	0.69
8	0.9	0.79 – 1.02	1.56	1.48 − 1.63	0.15	0.59
9	0.93	0.84 – 1.02	1.26	1.20 − 1.31	0.11	0.58
10	−0.04	−0.11 − −0.02	0.99	0.94 − 1.03	0.09	0.6
11	0.52	0.45 − 0.59	0.86	0.81 – 0.91	0.1	0.58
Stratum 3						
1	0.32	0.25 − 0.39	1.49	1.37 − 1.61	0.24	0.65
2	−0.07	−0.09 − −0.05	0.67	0.59 − 0.76	0.17	0.7
3	0.18	0.15 − 0.22	0.98	0.89 −1.08	0.19	0.66
4	0.11	0.09 − 0.14	0.89	0.83 − 0.95	0.12	0.75
5	0.01	−0.05 − 0.07	1.35	1.24 − 1.46	0.22	0.66
6	0.34	0.27 − 0.40	1	0.89 −1.11	0.22	0.54
7	−0.35	−0.40 − −0.31	1.22	1.14 − 1.31	0.17	0.73
8	0.5	0.39 − 0.60	2.03	1.84 −2.23	0.39	0.6
9	0.45	0.36 − 0.53	1.63	1.48 − 1.78	0.3	0.62
10	−0.07	−0.09 − −0.05	0.76	0.68 − 0.85	0.17	0.67
11	0.19	0.14 − 0.25	1.21	1.11 − 1.31	0.2	0.64

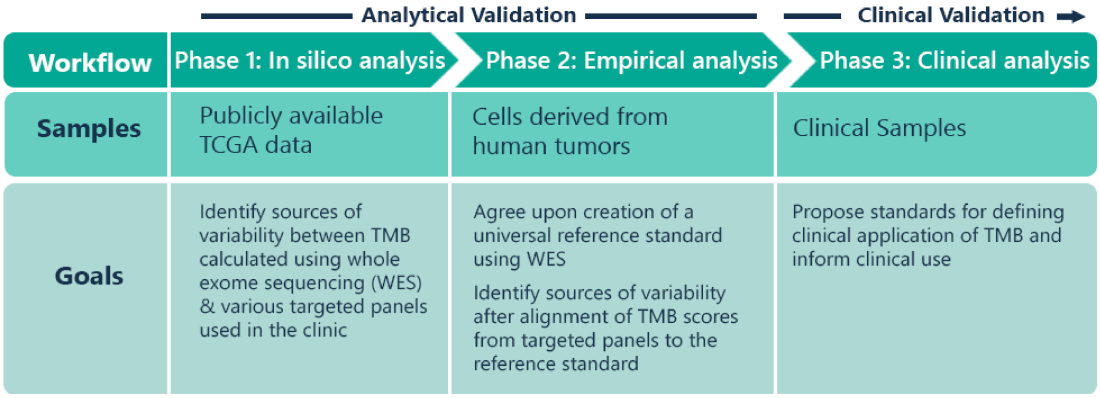
Abbreviations: CI, confidence intervals

Supplemental Table 4: Intercept and Slope Estimates for All Participating Laboratories, by Cancer Type in Stratum 1

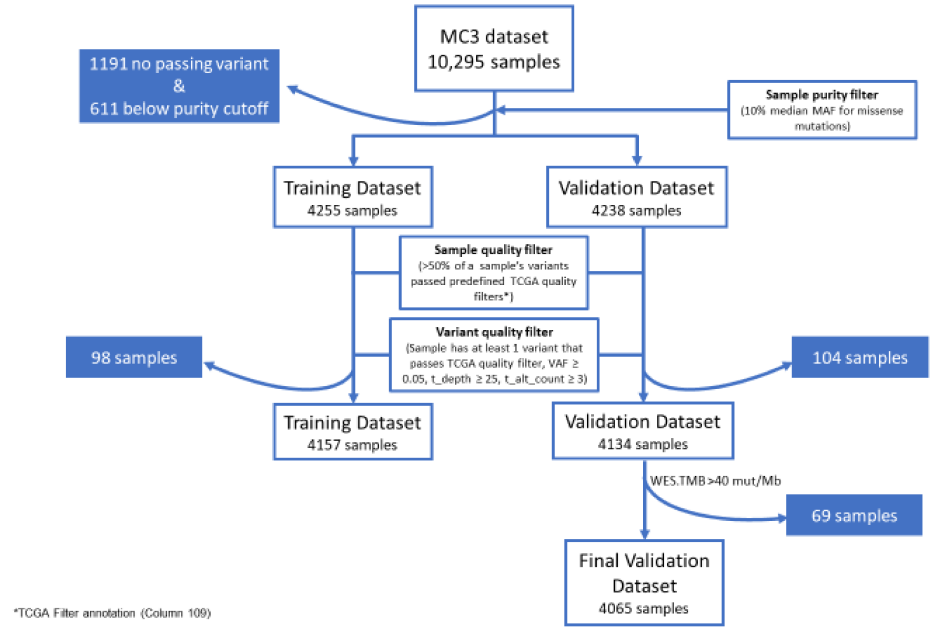
Cancer Type	Lab	Intercept	95% CI	Slope	95% CI	Δ
BLCA	1	0.598	(0.143-1.053)	1.398	(1.296-1.501)	0.205
	2	-0.345	(-0.676--0.015)	1.236	(1.161-1.311)	0.150
	3	0.163	(-0.219-0.544)	1.247	(1.158-1.336)	0.178
	4	-0.054	(-0.268-0.16)	1.042	(0.969-1.114)	0.145
	5	0.359	(-0.089-0.808)	1.251	(1.164-1.337)	0.173
	6	0.354	(-0.025-0.734)	1.065	(0.978-1.152)	0.174
	7	-0.162	(-0.476-0.153)	1.193	(1.117-1.268)	0.151
	8	0.869	(0.278-1.459)	1.790	(1.641-1.94)	0.299
	9	1.011	(0.513-1.509)	1.480	(1.36-1.6)	0.240
	10	-0.253	(-0.583-0.077)	1.178	(1.091-1.265)	0.174
	11	0.403	(0.05-0.755)	1.084	(1.004-1.164)	0.160
COAD	1	1.735	(1.34-2.13)	1.138	(1.057-1.22)	0.163
	2	0.191	(-0.167-0.548)	0.983	(0.889-1.077)	0.188
	3	1.215	(0.78-1.65)	1.037	(0.935-1.138)	0.203
	4	-0.064	(-0.321-0.194)	0.973	(0.894-1.052)	0.158
	5	0.662	(0.22-1.104)	1.027	(0.928-1.126)	0.198
	6	1.355	(0.97-1.739)	0.750	(0.659-0.84)	0.181
	7	-0.092	(-0.422-0.239)	1.058	(0.975-1.141)	0.166
	8	2.459	(1.804-3.114)	1.486	(1.346-1.625)	0.279
	9	2.216	(1.702-2.729)	1.275	(1.161-1.389)	0.228
	10	1.007	(0.569-1.446)	0.992	(0.892-1.091)	0.199
	11	0.897	(0.487-1.306)	1.059	(0.953-1.165)	0.212
HNSC	1	1.358	(1.018-1.698)	1.101	(0.987-1.215)	0.228
	2	-0.128	(-0.42-0.164)	1.129	(1.017-1.241)	0.224
	3	0.595	(0.257-0.933)	0.998	(0.885-1.11)	0.225
	4	0.303	(0.096-0.51)	0.854	(0.774-0.934)	0.160
	5	0.756	(0.403-1.108)	1.111	(0.997-1.226)	0.229
	6	0.802	(0.489-1.115)	0.890	(0.796-0.985)	0.189
	7	0.093	(-0.18-0.366)	1.012	(0.918-1.106)	0.188
	8	1.882	(1.335-2.43)	1.244	(1.092-1.395)	0.303
	9	1.948	(1.452-2.443)	1.163	(1.008-1.318)	0.310
	10	0.424	(0.077-0.77)	0.926	(0.816-1.035)	0.219
	11	0.693	(0.393-0.993)	0.919	(0.831-1.008)	0.177
LUAD	1	1.140	(0.848-1.432)	0.976	(0.925-1.027)	0.102
	2	-0.198	(-0.451-0.055)	1.075	(1.025-1.126)	0.101
	3	0.297	(0.057-0.538)	0.964	(0.91-1.018)	0.108
	4	0.227	(0.057-0.398)	0.817	(0.773-0.86)	0.087
	5	0.142	(-0.145-0.428)	1.135	(1.078-1.192)	0.114
	6	0.532	(0.241-0.822)	0.839	(0.792-0.886)	0.094
	7	0.199	(-0.033-0.431)	0.899	(0.854-0.945)	0.091
	8	2.103	(1.606-2.599)	1.095	(1.012-1.177)	0.165
	9	1.509	(1.157-1.861)	0.949	(0.888-1.01)	0.122
	10	0.173	(-0.089-0.435)	0.878	(0.829-0.926)	0.097
	11	0.667	(0.397-0.936)	0.828	(0.777-0.88)	0.103
LUSC	1	1.398	(0.846-1.950)	0.955	(0.875-1.035)	0.160
	2	0.405	(-0.093-0.903)	0.989	(0.916-1.062)	0.146
	3	0.745	(0.259-1.231)	0.901	(0.828-0.973)	0.145
	4	0.108	(-0.233-0.449)	0.800	(0.739-0.861)	0.122
	5	0.999	(0.395-1.604)	1.013	(0.916-1.11)	0.194
	6	1.214	(0.745-1.684)	0.741	(0.672-0.81)	0.138
	7	0.416	(-0.035-0.867)	0.869	(0.801-0.937)	0.136
	8	2.136	(1.349-2.923)	1.099	(0.99-1.209)	0.219
	9	1.743	(1.076-2.411)	0.950	(0.857-1.043)	0.186
	10	0.422	(-0.06-0.904)	0.851	(0.778-0.925)	0.147
	11	0.584	(0.059-1.108)	0.896	(0.817-0.974)	0.157
SKCM	1	0.892	(0.486-1.298)	0.908	(0.86-0.955)	0.095
	2	-0.426	(-0.646--0.206)	1.123	(1.072-1.174)	0.102
	3	0.076	(-0.213-0.366)	1.006	(0.961-1.051)	0.09
	4	0.056	(-0.104-0.217)	0.883	(0.842-0.924)	0.082
	5	0.183	(-0.196-0.563)	0.961	(0.911-1.011)	0.100
	6	0.773	(0.381-1.165)	0.772	(0.728-0.817)	0.089
	7	0.011	(-0.277-0.298)	0.913	(0.873-0.953)	0.08
	8	1.573	(0.924-2.223)	1.213	(1.146-1.28)	0.134
	9	1.221	(0.777-1.665)	0.933	(0.876-0.99)	0.114
	10	-0.227	(-0.468-0.015)	0.998	(0.939-1.058)	0.119
	11	0.616	(0.298-0.933)	0.843	(0.792-0.894)	0.102
STAD	1	0.337	(0.046-0.629)	1.227	(1.172-1.282)	0.110
	2	-0.095	(-0.377-0.187)	1.022	(0.956-1.087)	0.131
	3	-0.069	(-0.369-0.231)	1.085	(1.02-1.15)	0.130
	4	-0.116	(-0.31-0.077)	0.961	(0.908-1.014)	0.106
	5	0.496	(0.156-0.835)	1.104	(1.037-1.17)	0.133
	6	0.681	(0.419-0.943)	0.734	(0.684-0.783)	0.099
	7	-0.002	(-0.258-0.254)	1.015	(0.959-1.071)	0.112
	8	0.543	(0.092-0.994)	1.405	(1.307-1.503)	0.196
	9	0.985	(0.59-1.38)	1.197	(1.121-1.273)	0.152
	10	-0.225	(-0.508-0.058)	1.009	(0.944-1.073)	0.129
	11	0.107	(-0.175-0.389)	1.060	(1-1.121)	0.121
UCEC	1	2.066	(1.749-2.382)	1.249	(1.19-1.308)	0.118
	2	0.240	(-0.019-0.498)	1.055	(0.988-1.123)	0.135
	3	1.279	(0.979-1.578)	1.263	(1.191-1.334)	0.143
	4	0.452	(0.285-0.618)	1.073	(1.019-1.127)	0.108
	5	0.782	(0.501-1.063)	1.088	(1.029-1.147)	0.118
	6	1.656	(1.413-1.900)	0.815	(0.747-0.883)	0.136
	7	0.275	(0.047-0.503)	1.113	(1.055-1.172)	0.117
	8	2.860	(2.359-3.361)	1.602	(1.49-1.714)	0.224
	9	2.633	(2.271-2.995)	1.392	(1.317-1.467)	0.150
	10	1.027	(0.732-1.321)	1.120	(1.055-1.185)	0.130
	11	1.256	(0.950-1.561)	0.755	(0.655-0.855)	0.200

Abbreviations: CI, confidence intervals; Δ, delta, or difference between slope's upper limit and lower limit for each laboratory

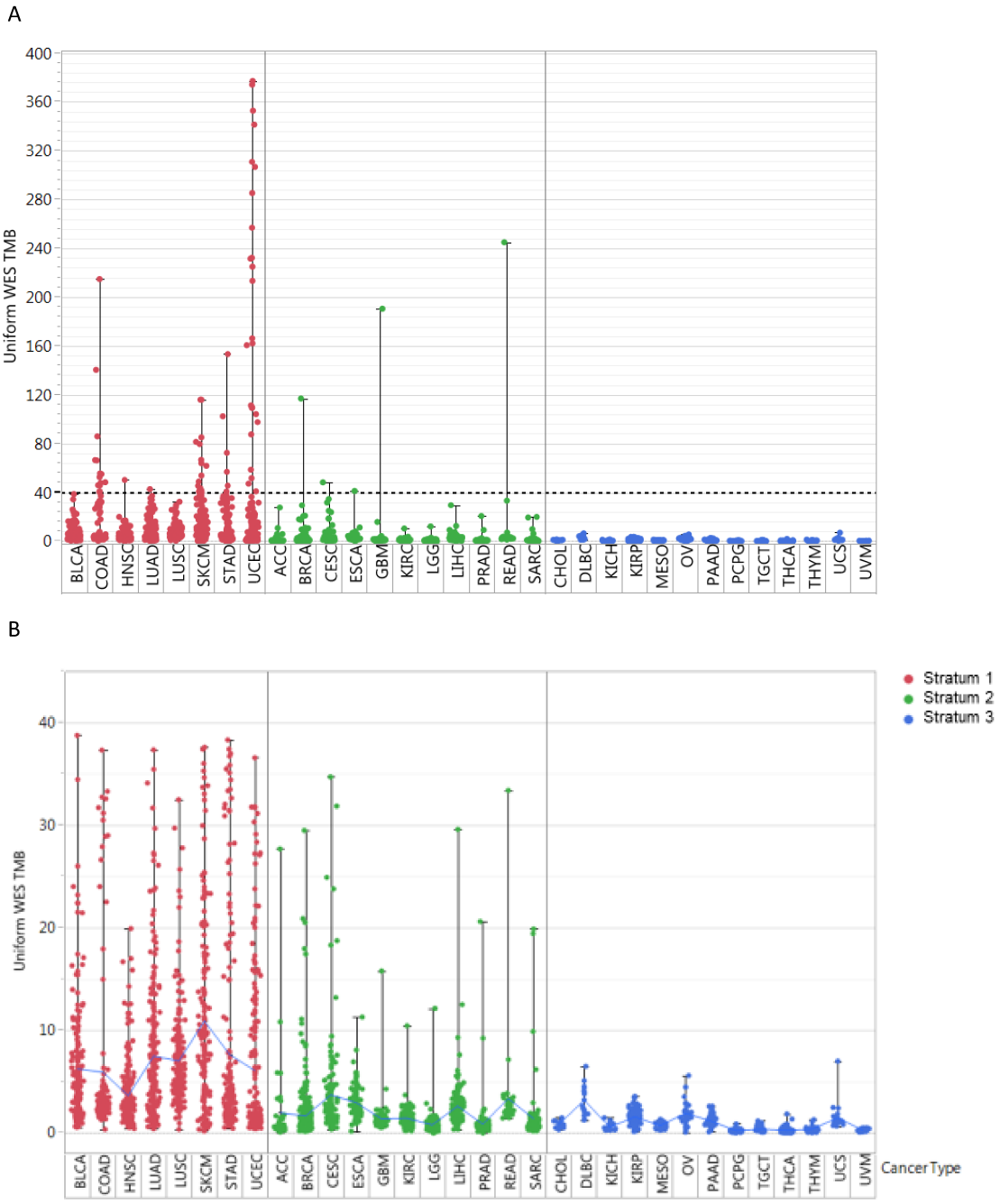
SUPPLEMENTAL FIGURES:



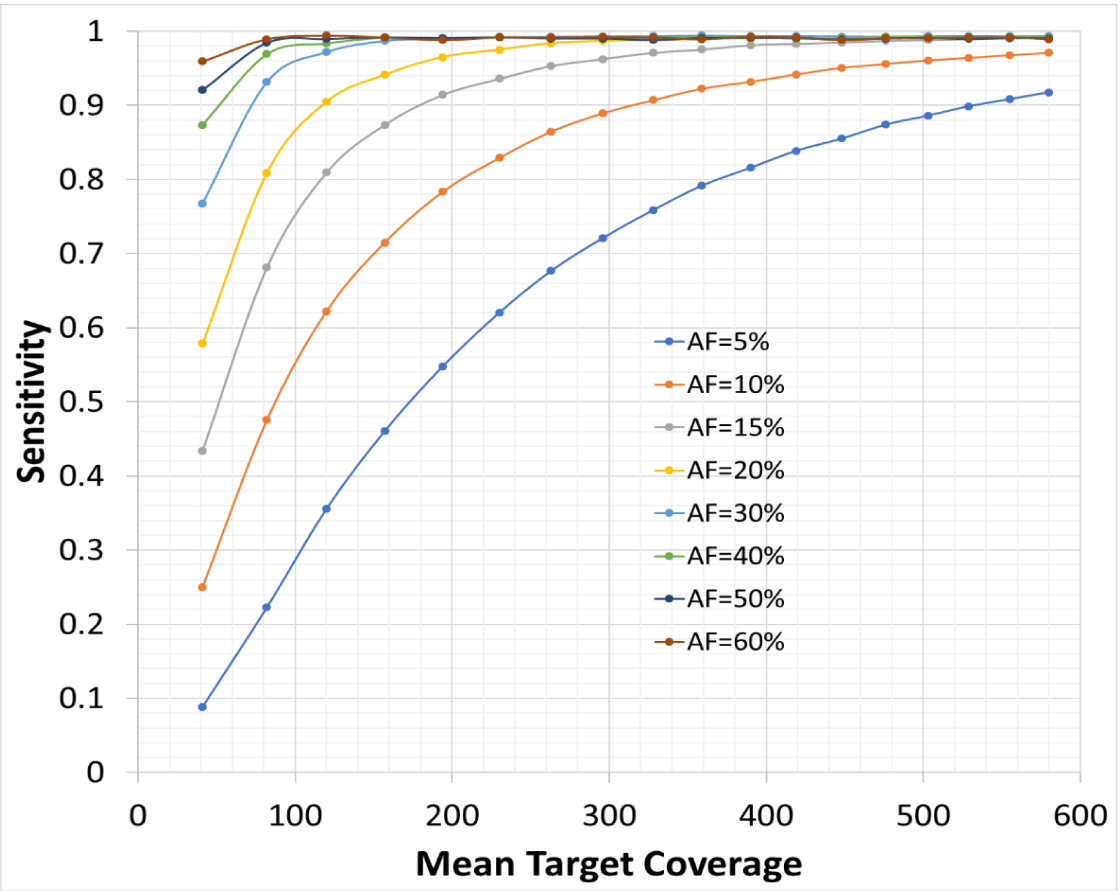
Supplemental Figure 1. Friends of Cancer Research Tumor Mutational Burden (TMB) Harmonization Project overview. Abbreviations: TCGA, the Cancer Genome Atlas; TMB, tumor mutational burden; WES, whole exome sequencing



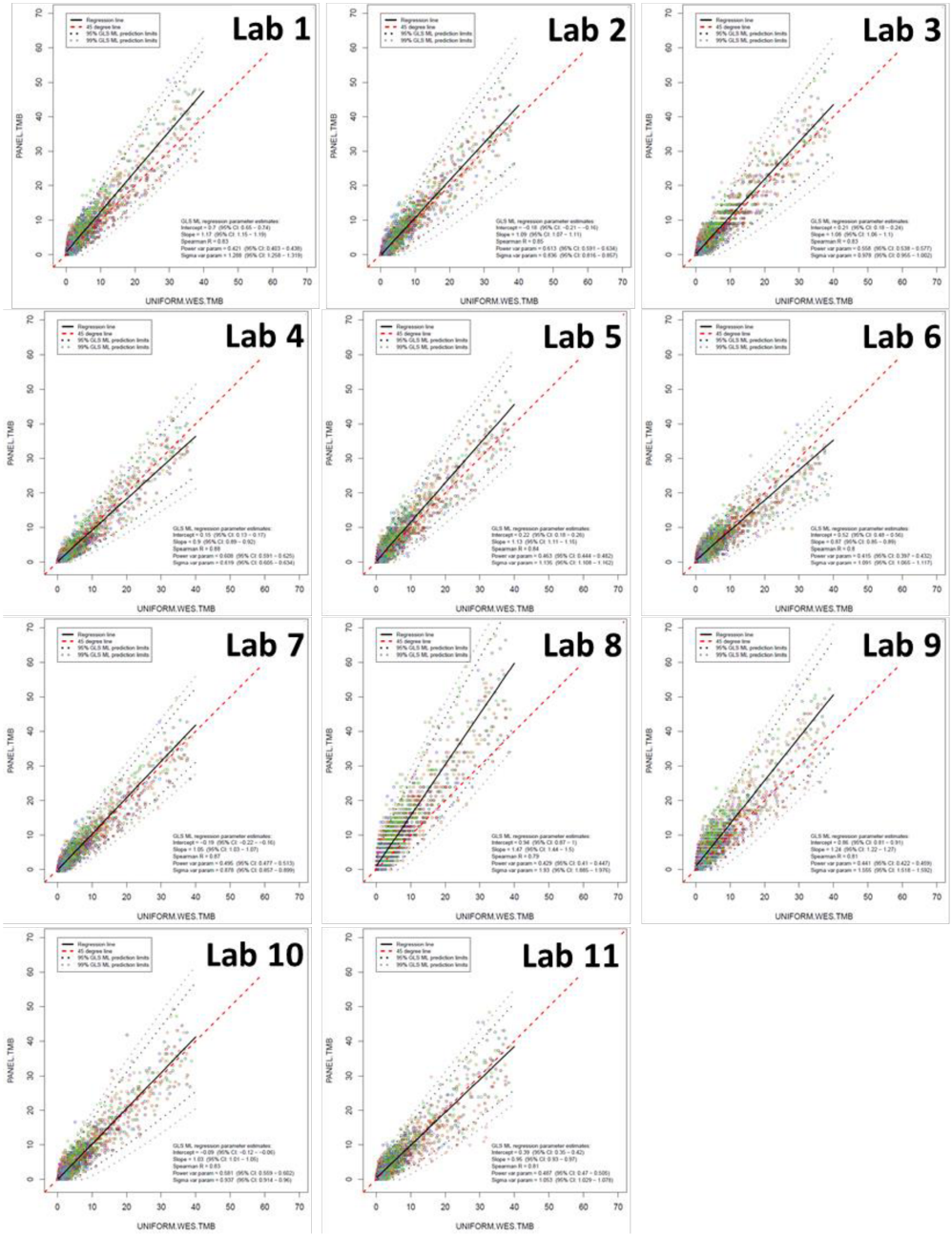
Supplemental Figure 2: Flowchart depicting the filtering methods used to derive the final validation dataset used in the study



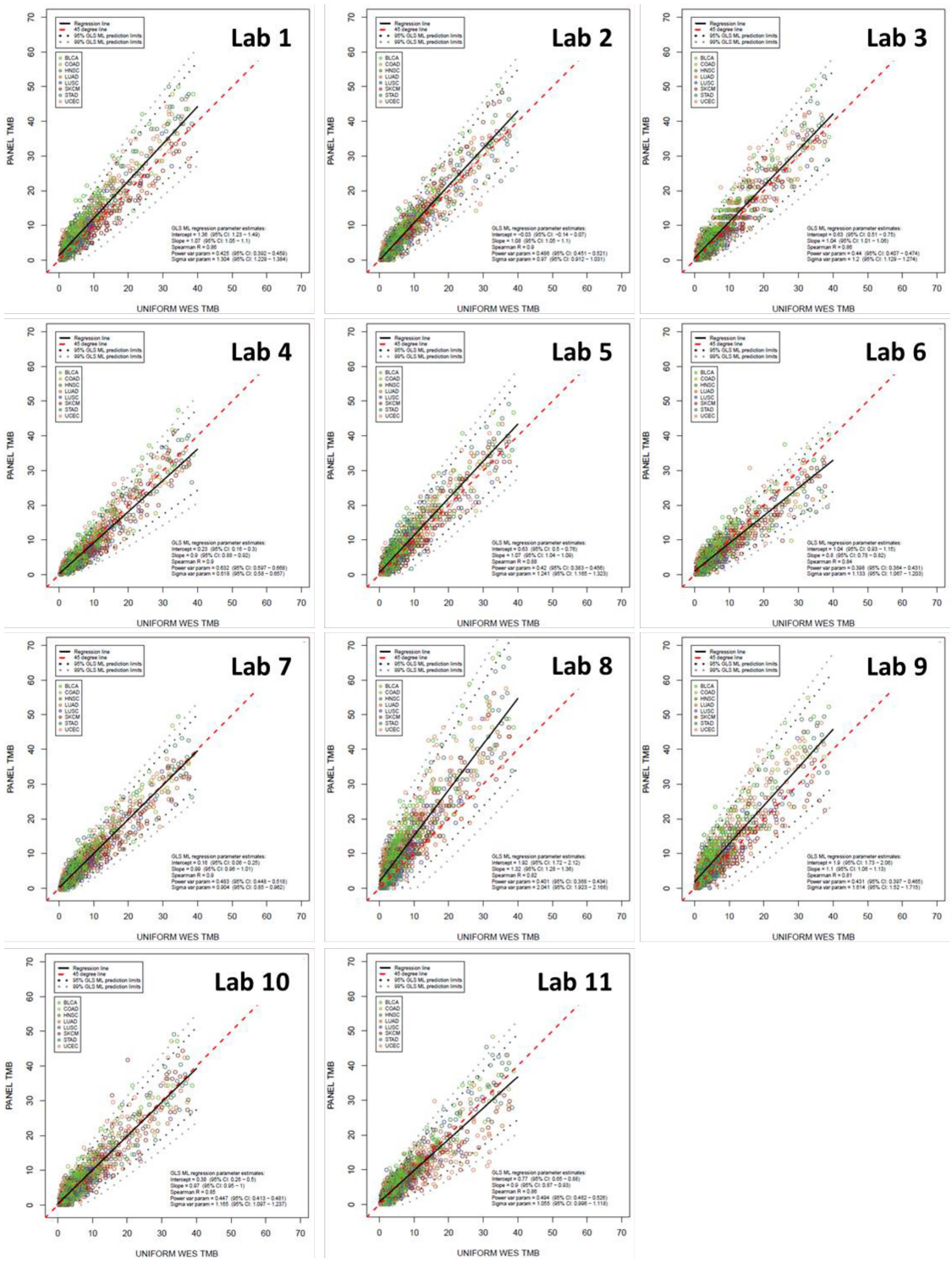
Supplemental Figure 3: A. Distribution of WES TMB values per cancer type divided by stratum, B. Distribution of WES TMB values (capped at 40 mut/Mb) per cancer type divided by stratum



Supplemental Figure 4: Sensitivity at various target coverage values by allele frequency. Two contrived samples (mixes of 10 different HapMap cell lines) were created as described by Frampton et.al. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5710001/) to create a pool of samples with variant allele frequencies ranging between 5% to 100%. These were sequenced using Agilent's SureSelect platform (v5) on the NovaSeq sequencing system generating 150bp paired-end reads. Reads were aligned to the hg19 reference build, generating >600X coverage for the targeted region of the exome (~80MB). A gold standard was also created for these samples by sequencing each cell line individually. The contrived samples were compared against the gold standard. At this high sequencing depth, the sensitivity achieved is >97% for variants with ≥10% VAF and >91% for variants ≥5% VAF. To check the sensitivity as a function of depth, these samples were down-sampled to FASTQ level using The Seqtk tool for 90%-5% of the original reads generating samples with median target coverage from ~580X to 40X. This plot shows the performance of a particular dilution for different variant allele frequency variants against the gold standard. 300X depth for an exome captures all the variants with >72% sensitivity and variants with ≥10% variant allele frequency at sensitivity >90%.

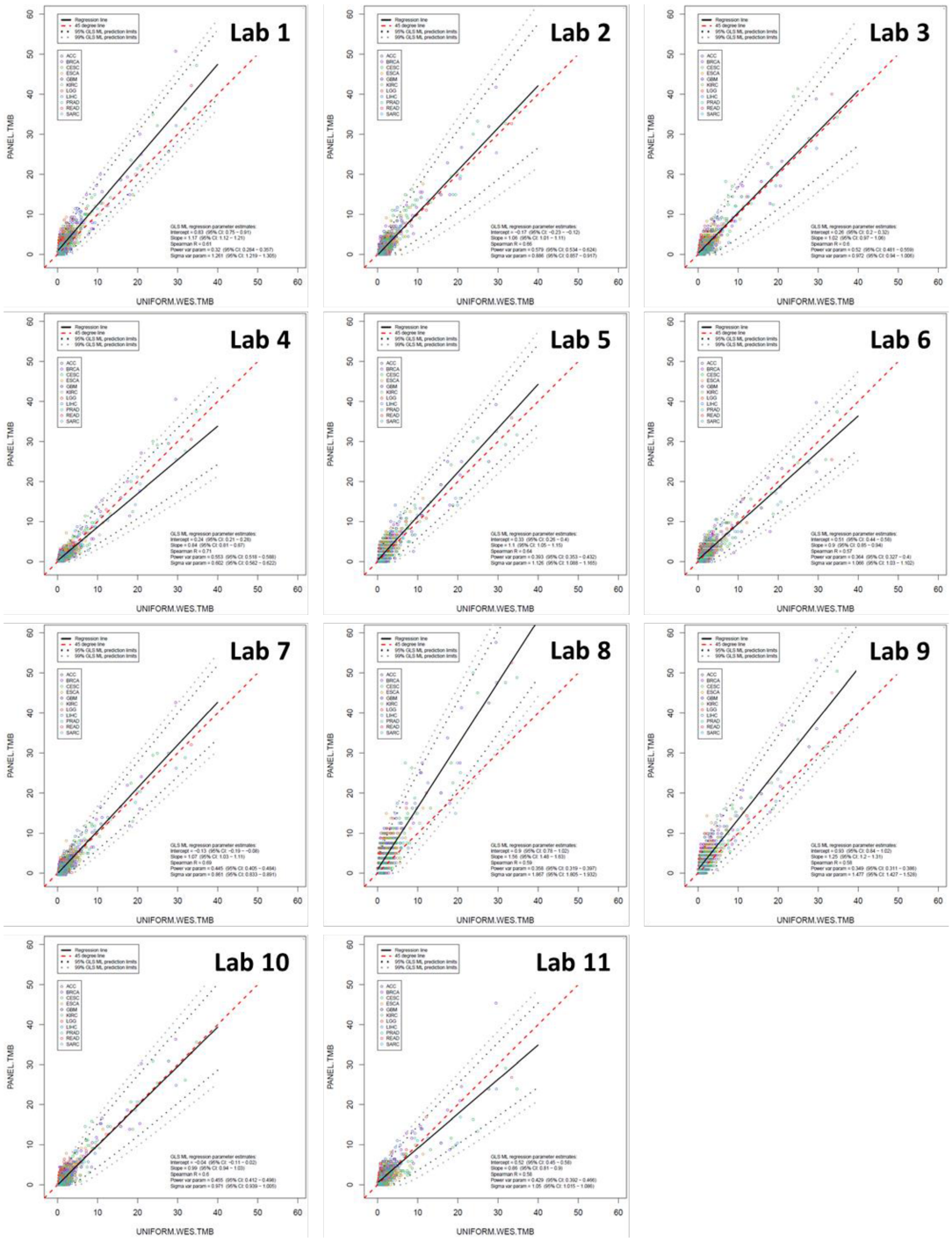


Supplemental Figure 5: Scatterplot for panel TMB as a function of WES TMB for each participating laboratory for all cancer types in the validation dataset of the TCGA MC3 dataset. The black solid line represents the estimated regression line, and the red dashed line represents the 45° line. The dark gray and light gray dotted lines represent 95% and 99% prediction limits calculated from the fitted weighted regression models.


Lab 1
Lab 2
Lab 3
Lab 4
Lab 5
Lab 6
Lab 7
Lab 8
Lab 9
Lab 10
Lab 11
PANEL TMB
UNIFORM WES TMB
Regression line
45 degree line
95% GLS ML prediction limits
99% GLS ML prediction limits
BLCA
COAD
HNSC
LUAD
LUSC
SKCM
STAD
UCEC

Supplemental Figure 6: Scatterplot for panel TMB as a function of WES TMB for each participating laboratory for only those cancer types in stratum 1 (most cancer types span the full range of 0-40mut/Mb). The black solid line represents the estimated regression line, and the red dashed line represents the 45° line. The dark gray and light gray dotted lines represent 95% and 99% prediction limits calculated from the fitted weighted regression models. Each color represents a different cancer type.

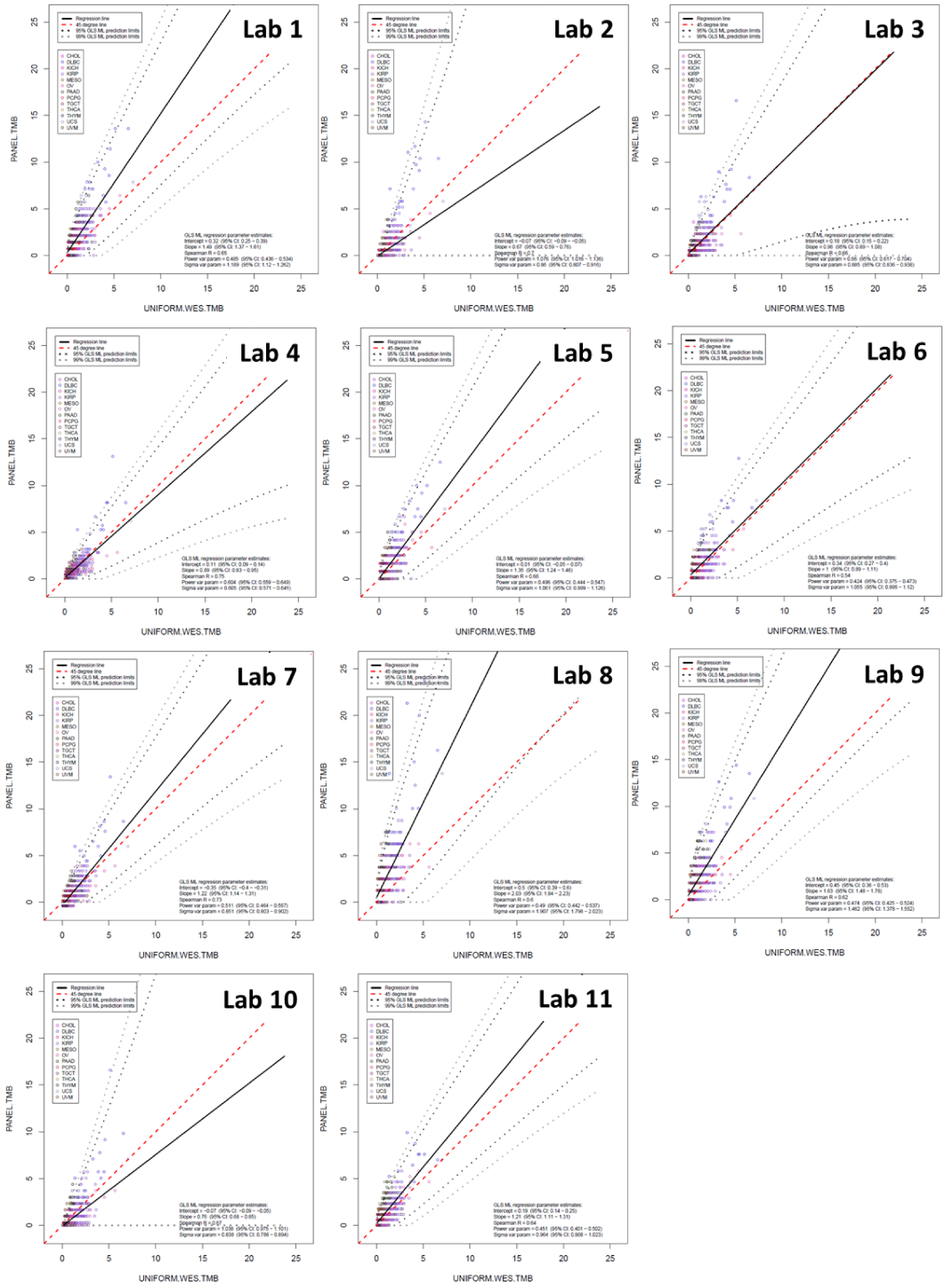
Merino DM, *et al. J Immunother Cancer* 2020; 8:e000147. doi: 10.1136/jitc-2019-000147



Supplemental Figure 7: Scatterplot for panel TMB as a function of WES TMB for each participating laboratory for only those cancer types in stratum 2 (most samples had low TMB values with occasional samples with values in the upper range, < 10 samples with TMB 10-40mut/Mb). The black solid line

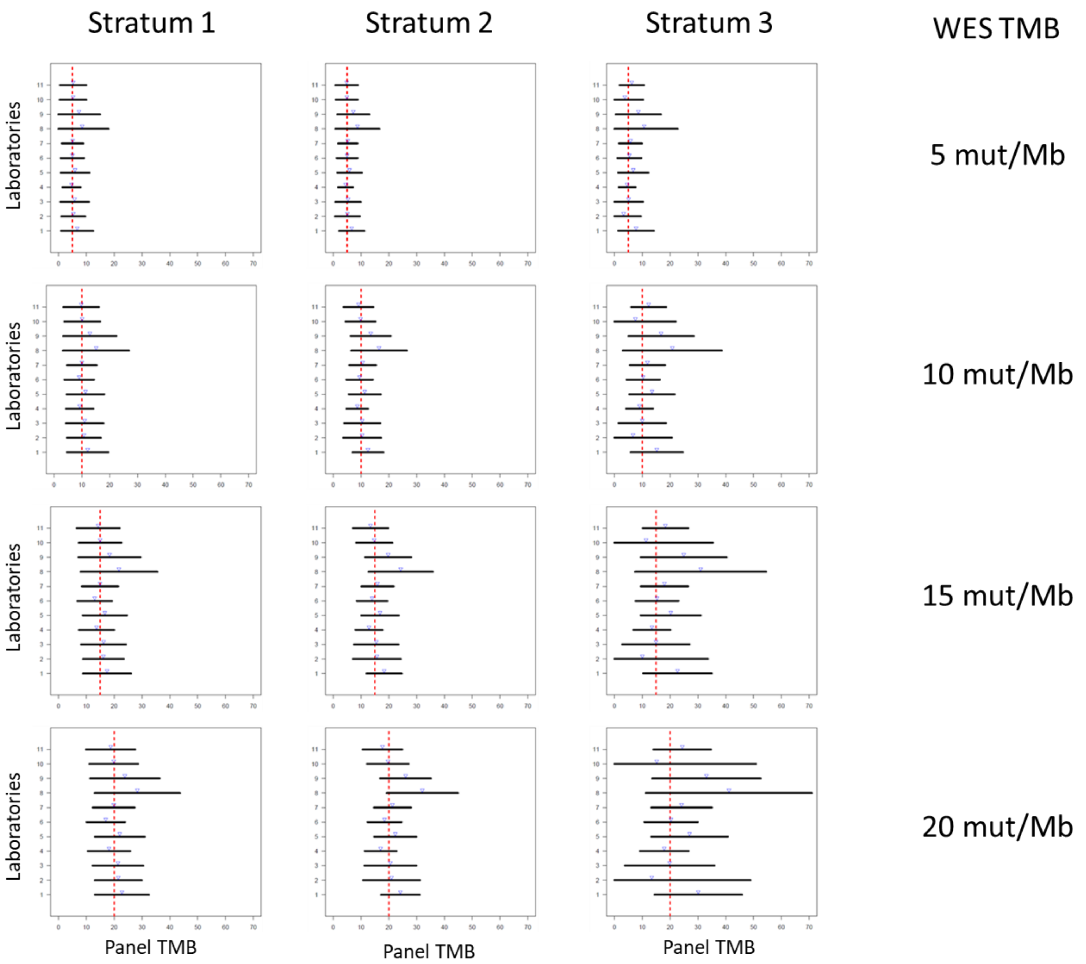
represents the estimated regression line, and the red dashed line represents the 45° line. The dark gray and light gray dotted lines represent 95% and 99% prediction limits calculated from the fitted weighted regression models.

Merino DM, *et al. J Immunother Cancer* 2020; 8:e000147. doi: 10.1136/jitc-2019-000147

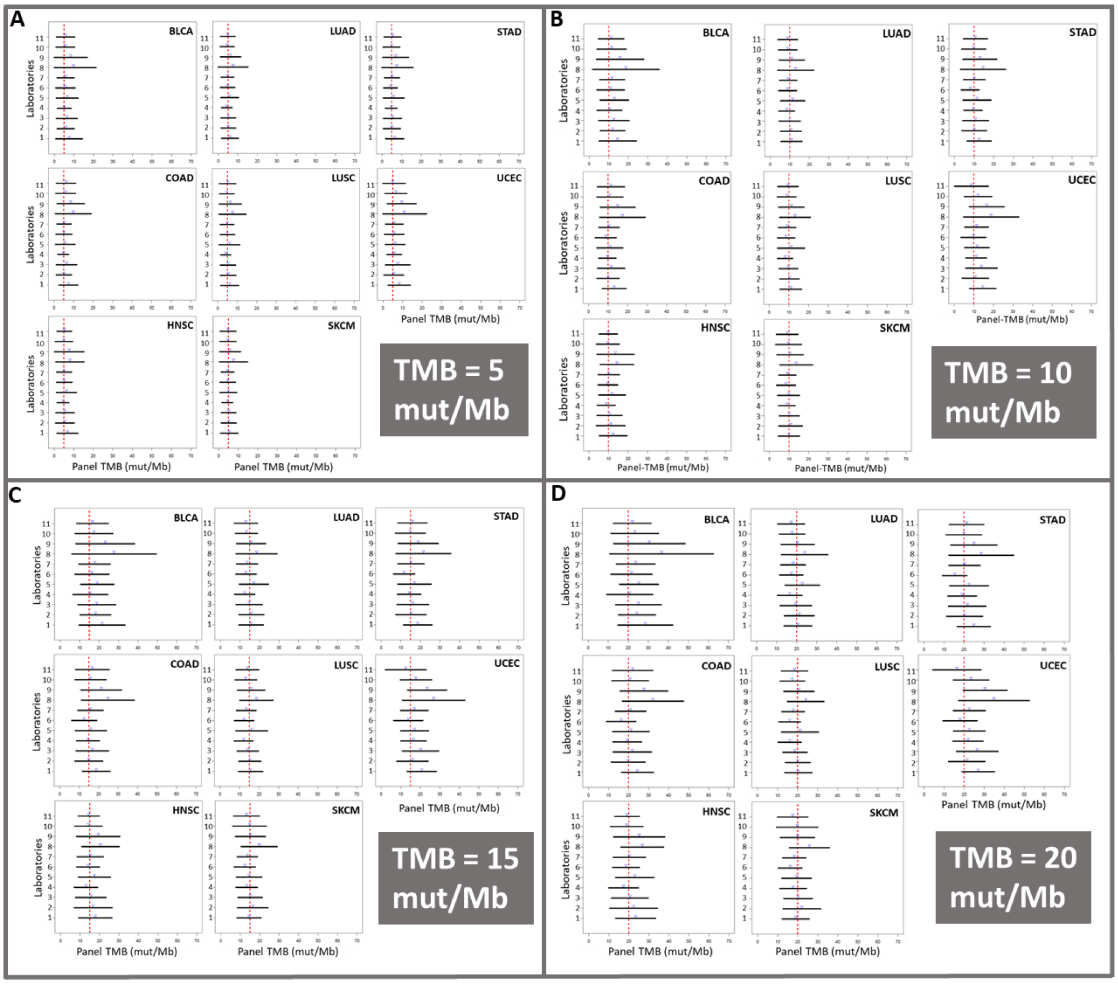


Supplemental Figure 8: Scatterplot for panel TMB as a function of WES TMB for each participating laboratory for only those cancer types in stratum 3 (most samples had low TMB values < 10 mut/Mb).

The black solid line represents the estimated regression line, and the red dashed line represents the 45° line. The dark gray and light gray dotted lines represent 95% and 99% prediction limits calculated from the fitted weighted regression models.



Supplemental Figure 9: 95% prediction intervals for panel TMB values estimated at discreet WES TMB values (5, 10, 15, and 20 mut/Mb), by stratum and laboratory. Blue arrows represent the estimated mean panel TMB for each laboratory. Red dashed line represents the discreet WES TMB value at which prediction intervals were calculated.



Supplemental Figure 10: 95% prediction intervals for panel TMB values estimated at discreet WES TMB values (Panels A: 5, B: 10, C: 15, and D: 20 mut/Mb), by cancer type and laboratory. Blue arrows represent the estimated mean panel TMB for each laboratory. Red dashed line represents the discreet WES TMB value at which prediction intervals were calculated.