Supplement Material for

Classifying cGAS-STING activity links chromosomal instability with immunotherapy response in metastatic bladder cancer

SUPPLEMENTARY TABLES

Supplementary Table 1: cGAS-STING interacting genes

Gene	Reference
STING1	[16], [17], [19], [28], [30], [48], [37], [18]
NFKB1	[16],[17],[28],[30],[37],[42]
IKBKG	[48]
TRAF6	[37,49]
RELA	[16],[17],[28],[30],[37]
NFKB2	[16],[17],[28],[37],[42],[48]
RELB	[16],[17],[28],[48],[37],[42]
MAP3K14	[18, 48]
CCL5	[19]
CXCL9	[16,19]
CXCL10	[16,19]
CXCL11	[19]
CGAS	[16],[17],[19],[28],[30]
ENPP1	[28]
TREX1	[37,48,49]
IFI16	[28],[48]
IRF3	[16],[17],[28],[30],[37],[49]
ATM	[18, 48]
TBK1	[17],[28],[30],[48],[37],[18]
PARP1	[18, 48]

Supplementary Table 2: Table showing the molecular characteristics of CSG1-4 in TCGA.

CSG	n	wGII	TMB	Responder genes	Activator genes	Responders-Activators ratio	Interpretation
1	361	0.41	4.58	0.04	3.62	0.001	Inactive
2	309	0.51	3.51	2.12	2.93	0.723	Non-functional
3	1048	0.46	5.16	5.16	4.41	1.17	Active
4	589	0.47	3.22	2.98	4.28	0.69	Abnormal activation

Supplementary Table 3: Table showing the summary of identified CSG in the BLCA immunotherapy cohort

CSG	n	wGII	TMB	Responder genes	Activator genes	IO Responders
1	59	0.31	0.66	0.50	2.86	3
2	65	0.39	1.68	0.93	2.49	18
3	138	0.42	1.73	2.55	3.25	33
4	86	0.39	1.19	1.31	3.16	14

Supplementary Table 4: Table showing estimated coefficients, their confidence intervals, hazard ratio and p-value of multivariate cox-proportional hazard model in immunotherapy treated cohort

	Pr(>|z|)	2.5 %	97.5 %	coef	exp(coef)
Stage II	**0.0203403748937784**	0.103194193813057	1.22670227074922	0.664948232281138	1.94438986210367
Stage III	**0.0190951008239468**	0.102769423905358	1.15266075046287	0.627715087184113	1.87332530039723
Stage IV	**0.00691135643931658**	0.210104970967461	1.32138876353428	0.765746867250868	2.15059998769121
CSG2	0.441871651781437	-1.96638443993066	0.85812418536040	-0.554130127285132	0.57457184824988
CSG3	0.297427390259272	-1.99564813060135	0.61027428260066	-0.692686924000341	0.50023018124695
CSG4	0.246936364549537	-0.60227020207462	2.34092209479392	0.869325946359645	2.3853024896969
wGII	0.444850517018845	-2.99078018926334	1.31306603791549	-0.838857075673923	0.43220421796213
TMB	0.0613319062308628	-1.90824464846835	0.04426547546405	-0.931989586502148	0.39376949202887
TIL	0.428140881579202	-1.05225883130894	0.44638499035084	-0.302936920479048	0.73864568831714
Gender = Male	0.864794010288844	-0.44893403915198	0.534359112728211	0.0427125367881154	1.04363784423917
CSG2 - wGII	0.911691703472188	-4.36608338041723	3.89843394249033	-0.23382471896345	0.79150053877235
CSG3 - wGII	0.907236972883025	-2.57797550547985	2.90388471381611	0.162954604168133	1.17698325834159
CSG4 - wGII	0.162014266915642	-7.3902653073123	1.23593468638121	-3.07716531046555	0.04608972169947
CSG2- TMB	0.126749414736743	-0.2335965136380	1.88154512643393	0.823974306397942	2.27954145474069
CSG3 - TMB	0.19361621395722	-0.42592427142464	2.10372884430321	0.838902286439286	2.3138256644108
CSG4 - TMB	0.382146243489956	-4.08394418534222	1.56505398657063	-1.25944509938579	0.28381146997199
wGII - TMB	**0.0191915532513094**	0.363234549626664	4.09230579524581	2.22777017243624	9.27915208466129
CSG2 - wGII- TMB	0.0701265343540397	-5.02205283119336	0.19825756127655	-2.4118976349584	0.08964501954049
CSG3 - wGII- TMB	**0.0443720743209857**	-4.81381779544736	-0.06134065225412	-2.43757922385074	0.0873721039666
CSG4 - wGII - TMB	0.531132206789143	-4.42498082079392	8.58078991890764	2.07790454905686	7.98771350356211

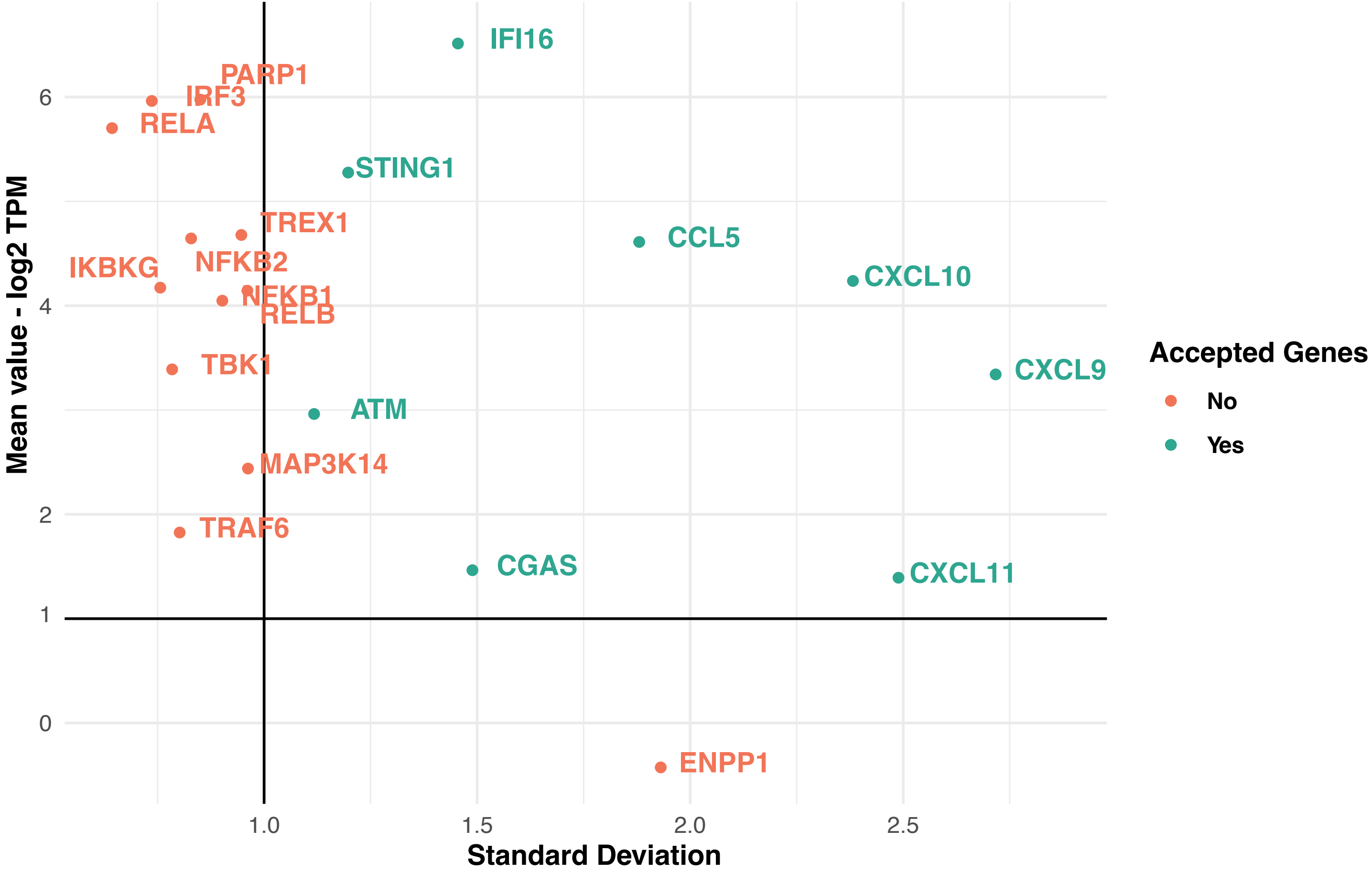


Figure S1. Scatter plot showing mean gene expression of the selected immune genes across the chosen cancer types (TCGA) versus the standard deviation. Values of "Yes" were used to identify genes with sufficient expression and high inter-sample variation.

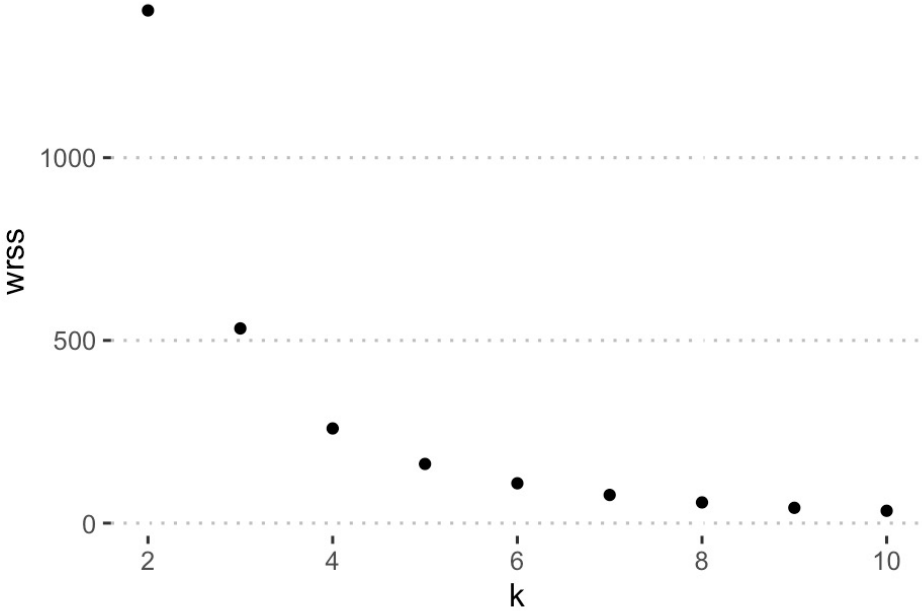


Figure S2. K-means elbow plot, showing Within-Cluster-Sum of Squared Errors (WSS) versus increasing values of K. Optimal number of clusters found at k= 4.

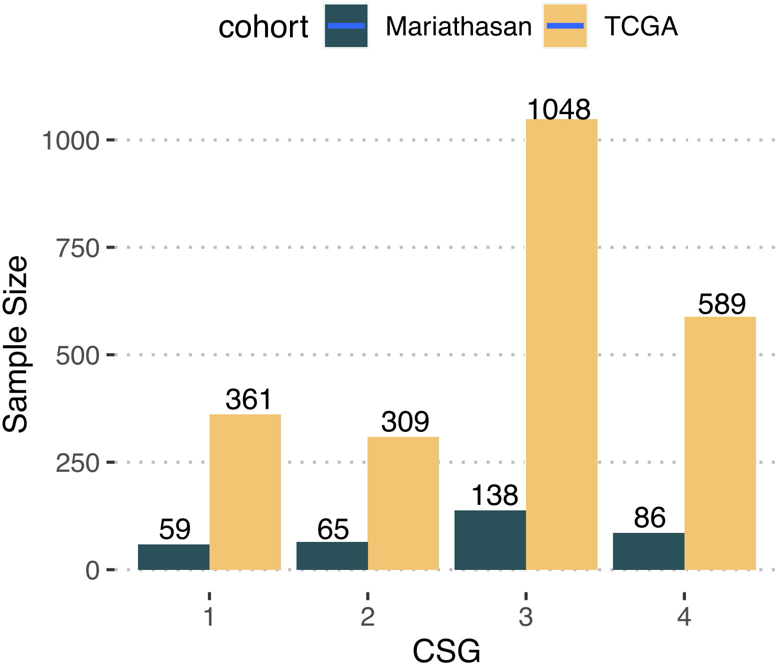


Figure S3. Sample sizes corresponding to each CSG and each cohort.

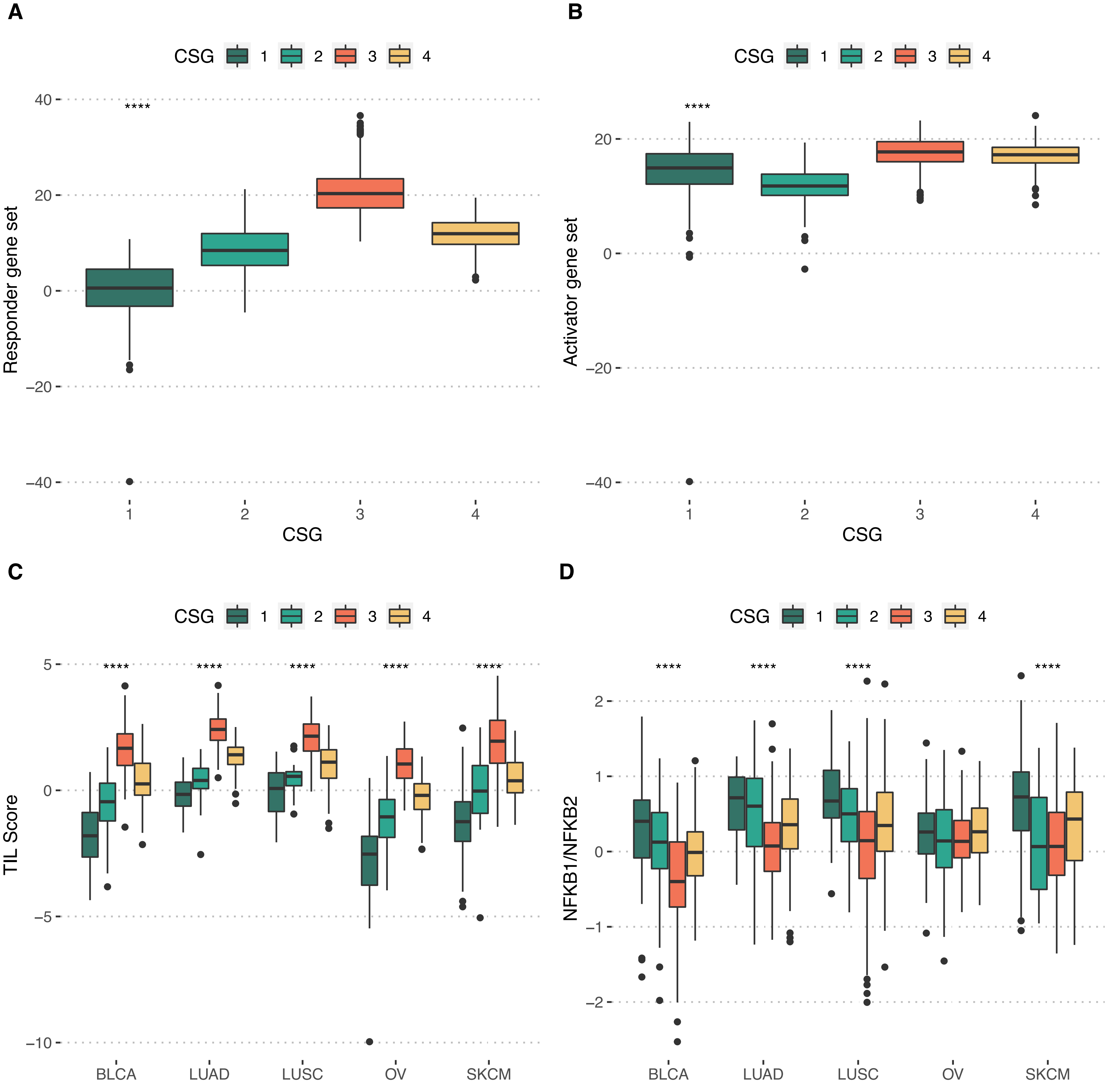


Figure S4. Mean expression of Activator (A) and Responder (B) genesets relative to CSG. (C) TIL score by CSG and by cancer type. (D) Gene expression ratio between NFKB1 and NFKB2 by CSG and by cancer type.

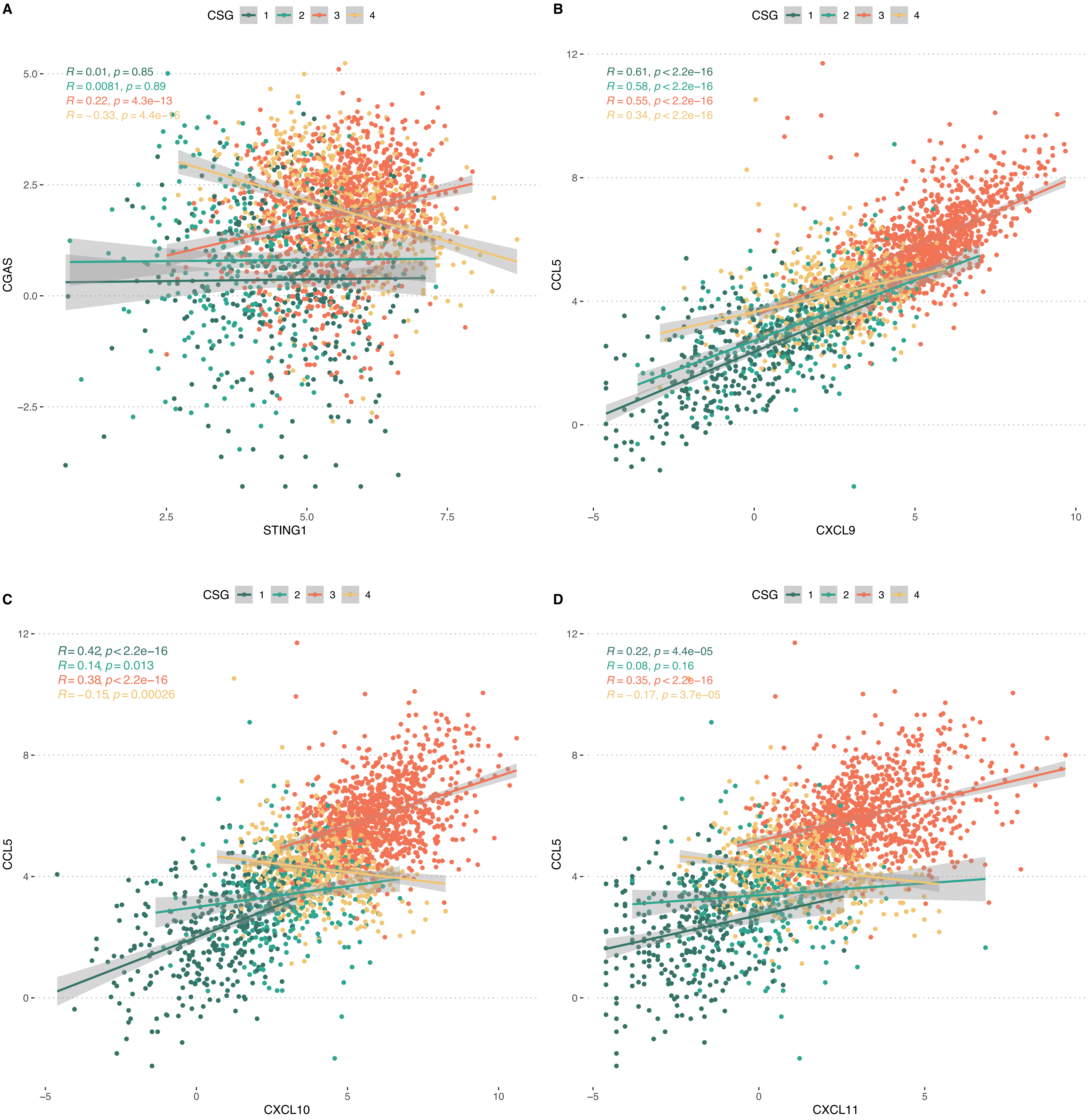


Figure S5. Scatter plots showing different correlation depending on CSG class between (A) Activator genes cGAS and STING1 within CSG 1–4, (B–D) Responder genes CCL5 and CXCL9, CXCL10 and CXCL11.

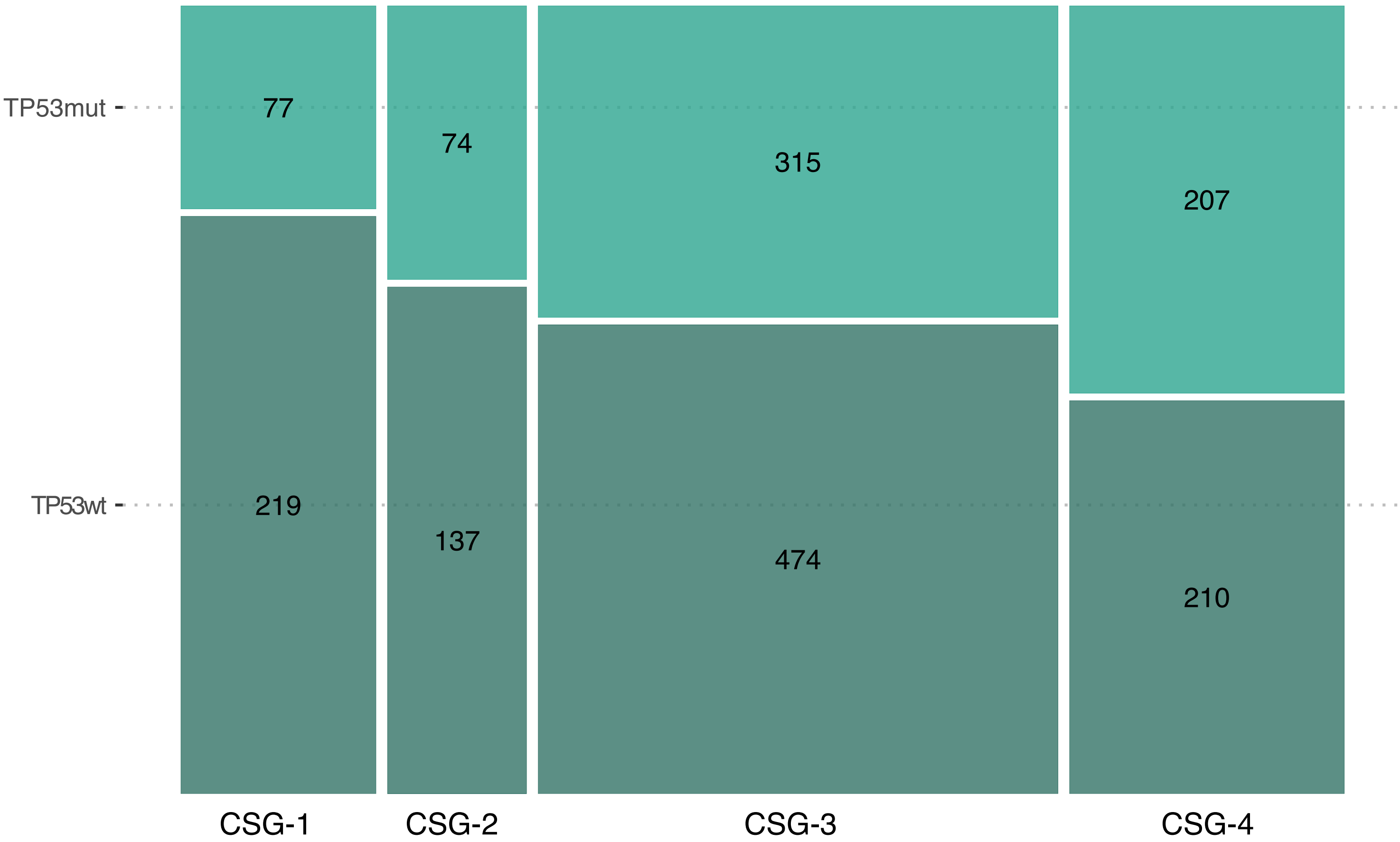
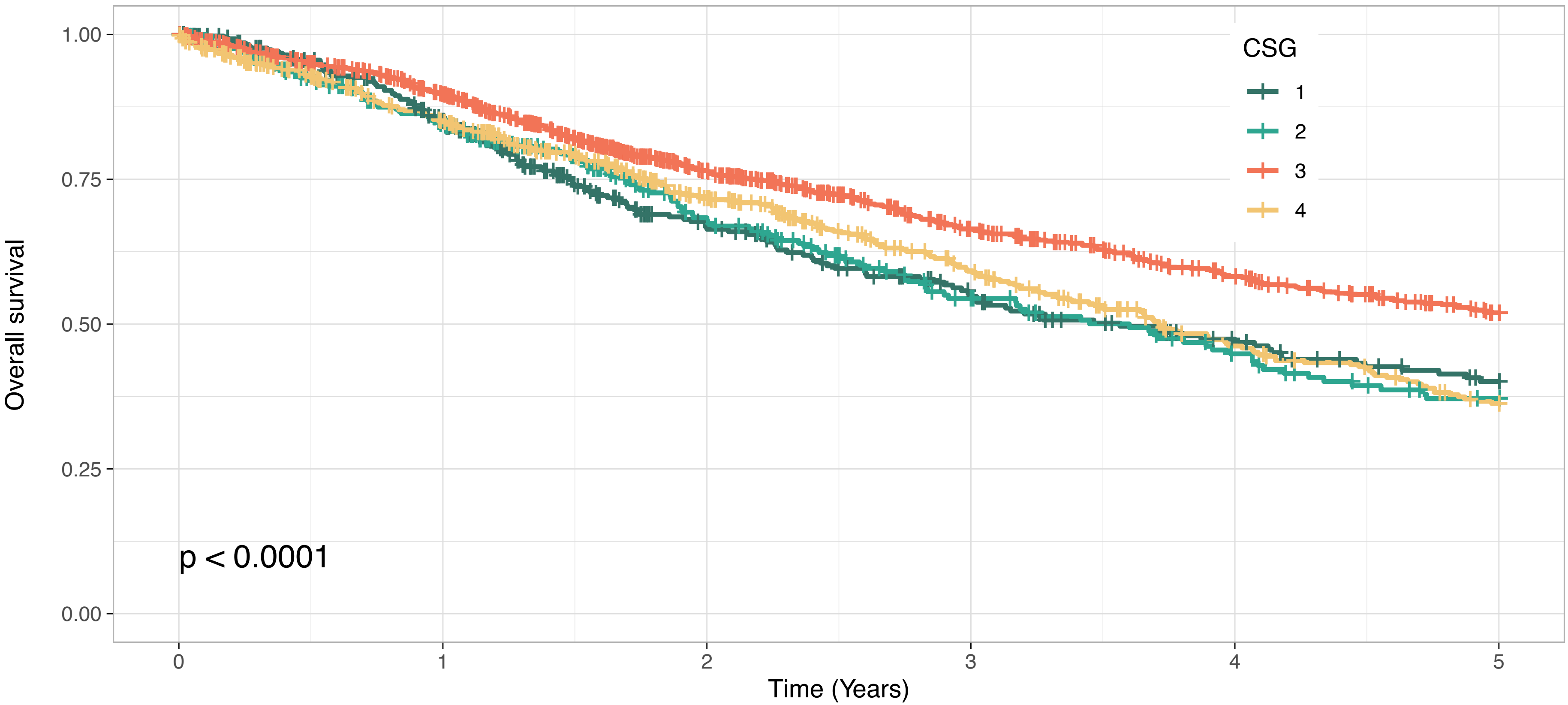


Figure S6. Distribution of samples with TP53 mutation by CSG across 2307 TCGA tumours



Number at risk: n (%)

CSG	0	1	2	3	4	5
🟩	351 (100)	266 (76)	156 (44)	110 (31)	82 (23)	61 (17)
🟦	308 (100)	228 (74)	142 (46)	90 (29)	67 (22)	48 (16)
🟥	1034 (100)	830 (80)	553 (53)	390 (38)	292 (28)	217 (21)
🟨	581 (100)	440 (76)	286 (49)	195 (34)	130 (22)	95 (16)

Time (Years)

Figure S7. Overall survival time within the combined 2307 TCGA samples representing patients with bladder, lung adeno, lung squamous cell, ovarian and melanoma cancer when stratified by CSG.

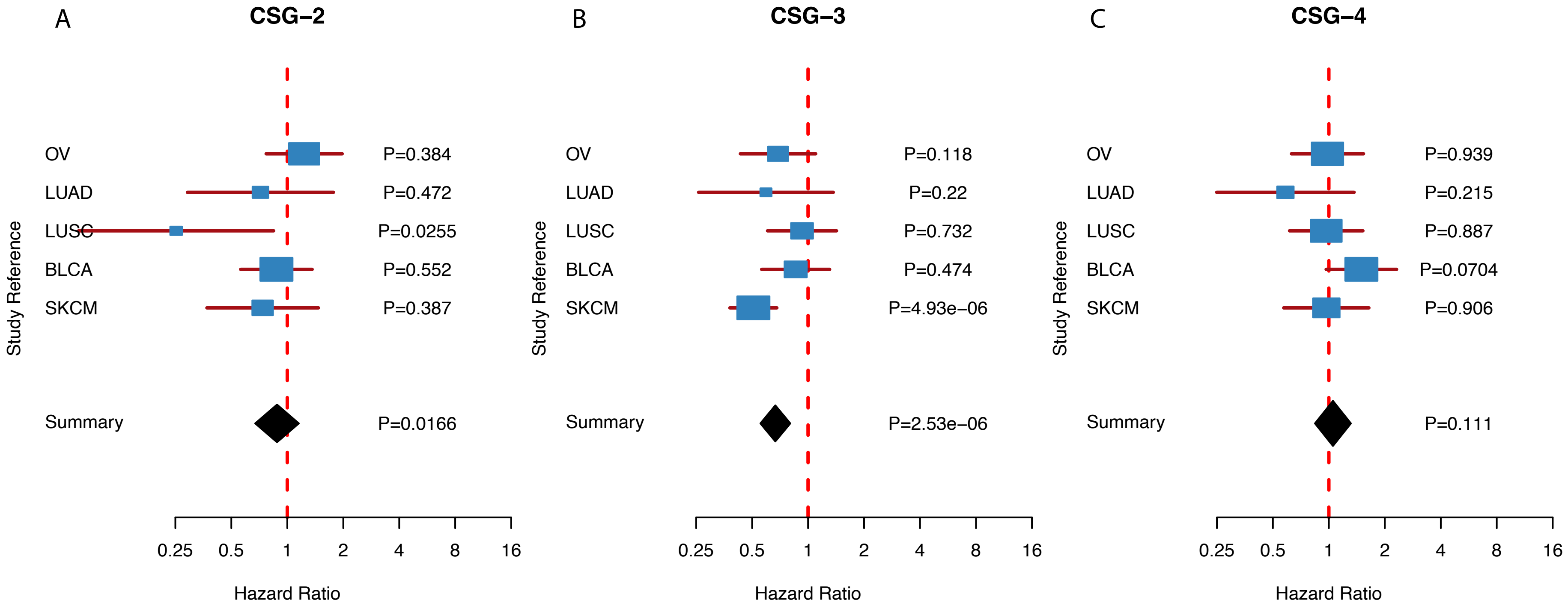


Figure S8. Meta-analysis of over-all survival time across bladder (BLCA), lung adeno (LUAD), lung squamous cell (LUSC), ovarian (OV), and melanoma (SKCM) samples, comparing CSG-1 against (A) CSG-2, (B) CSG-3, (C) CSG-4, respectively. This shows CSG-3 strongly associates with improved outcome. P-values for individual cancer types are based on Cox proportional hazard models. Summary P-value is calculated based on Fisher's method.

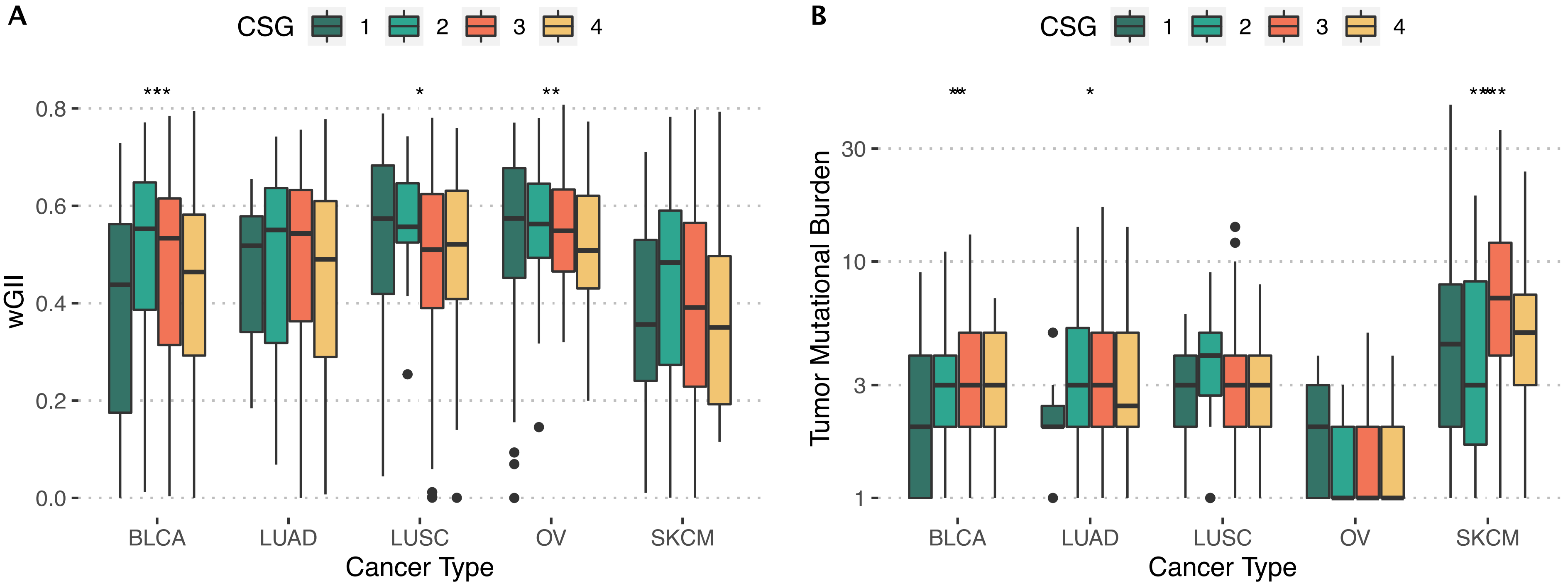


Figure S9. Distribution of (A) wGII and (B) tumour mutation burden by CSG and cancer type.
* indicates $p < 0.05$ within a given cancer type

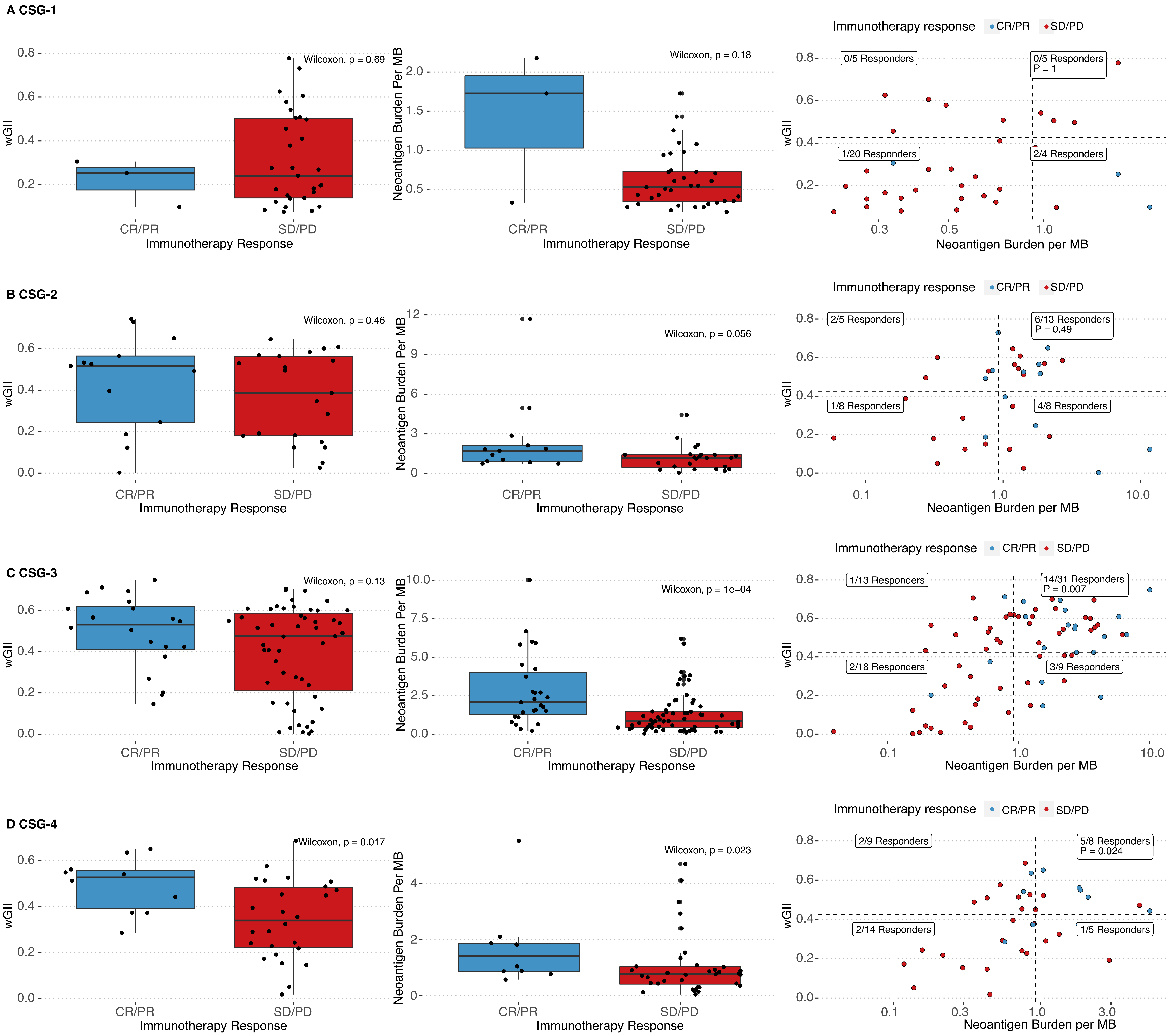


Figure S10. Immunotherapy response in the validation cohort, shown within CSG−1(A), CSG−2(B), CSG−3(C) and CSG−4(D). Each row shows binary therapy response against wGII and Neoantigen burden, and a scatter plot showing wGII versus neoantigen burden.
CR: Complete response. PR: Partial response. SD: Stable disease. PD: Progressive disease.

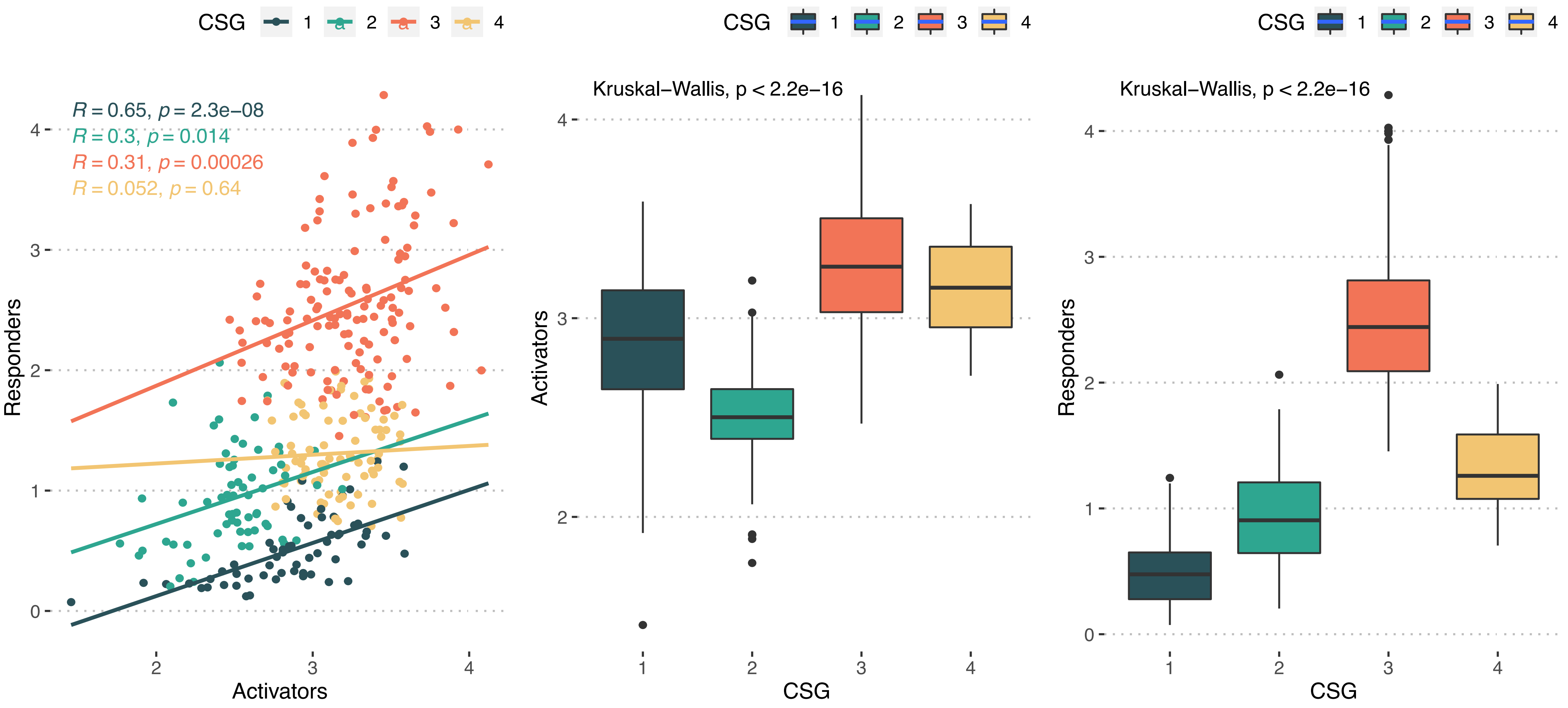


Figure S11. Analysis done on the test cohort indicates the difference between CSG-3 and CSG-4. CSG-3 shows positive correlation between mean expression of activator genes and mean value of responders genes (A). CSG-3 and CSG-4 show similar patterns in expression of activator genes (B) however responder genes are significantly lower in CSG-4 (C)

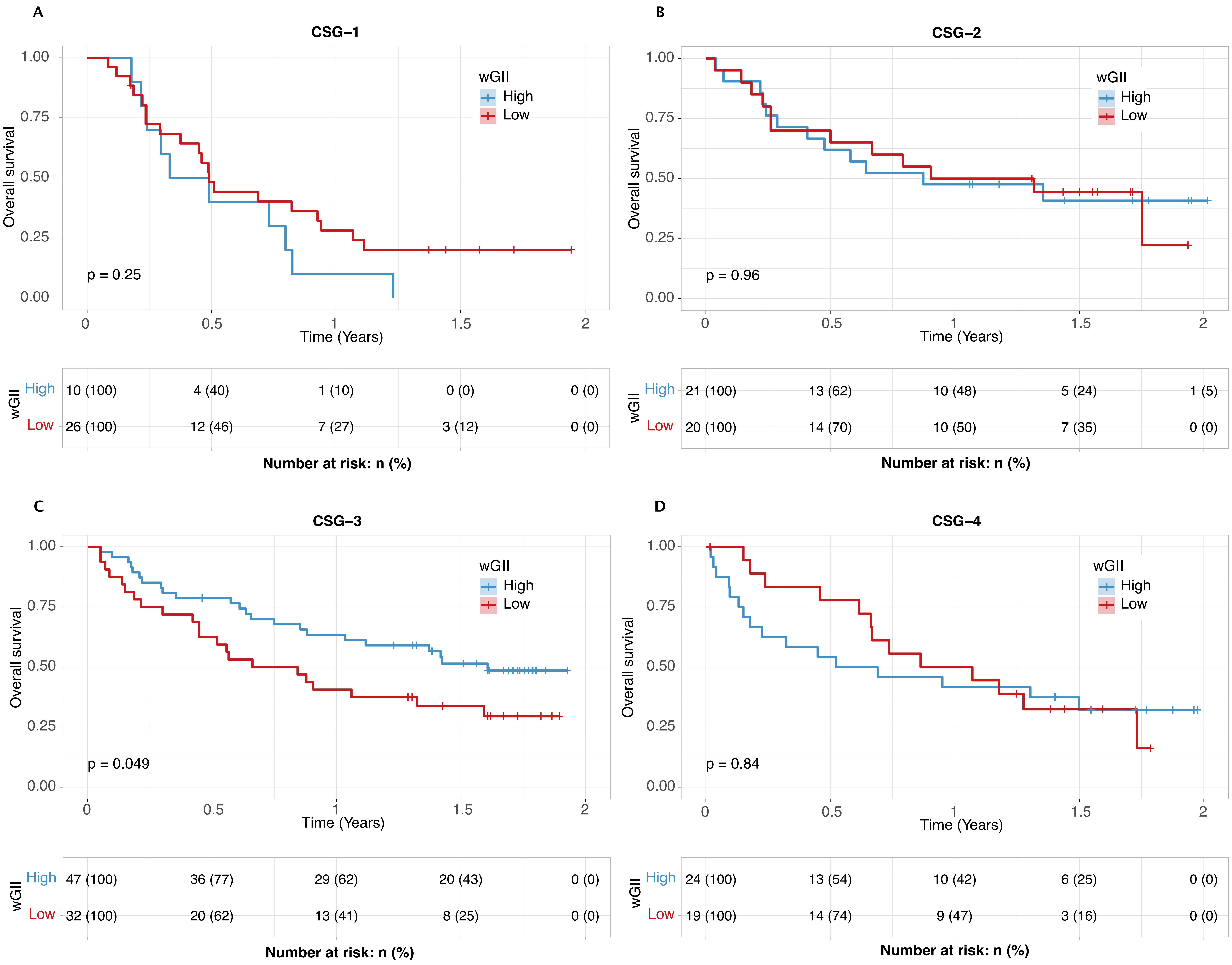


Figure S12. Kaplan–Meieranalysis of the validation cohort, showing overall survival by CSG 1–4when split into high and low wGII based on the median value of the cohort.