Lasting response by vertical inhibition with cetuximab and trametinib in *KRAS*-mutated colorectal cancer patient-derived xenografts

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Supplementary Figures and Tables

Legends:

Suppl. Table 1: Mutations detected by targeted sequencing used for model selection.
For an overview regarding wild-type status also see Suppl. Figure 1.

Suppl. Table 2: Mutations detected by exome sequencing and filtered by the Cancer
Mutation Census tier 1 to 3 criteria. See also Suppl. Figure 1 for pre-treatment mutation status determined via targeted sequencing for model selection.

Suppl. Table 3: Summary of gene expression data for each individual xenograft tumor
Analyzed and filtered for KEGG MAPK pathway genes (p 0.05). 5dCT – 5 days of treatment with cetuximab and trametinib, CTR – cetuximab and trametinib resistant tumor. (see separate Excel File).

Suppl. Table 4: CMS classification of untreated control tumors.

Suppl. Figure 1: Overview of targeted sequencing results
For mutational analysis, a gene panel with 48 genes (212 Amplicons) was utilized. The mutational status of the 19 models included in the study is depicted. For details regarding the variants found see Suppl. Table 1.

Suppl. Figure 2: Response data for different anti-EGFR antibodies and MEK inhibitors tested in BoC105 and BoC147.
Indicated numbers of BoC105 (A-C) and BoC147 (D-F) PDX tumors were treated with either mono or combination treatments. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$. PR, partial response; PD, progressive disease. Each # indicates the last point in time a tumor was measured and taken out therafter.

Suppl. Fig. 3: Growth curves and waterfall plots of additional PDX models
Relative growth curves are derived from mean values ± SEM (error bars). Each # represents a tumor that was taken out of the treatment cohort at the indicated time point either because the tumor reached the maximum size criteria or due to health issues of the animal. Vertical gray bars indicate the end of the primary observational period of 28 days. Waterfall plots show the response after 59 days of treatment or before the end of the experiment compared with tumor volume at baseline. Each bar represents one tumor. Dotted lines indicate the cut-

off values for progressive disease (PD) and partial response (PR). *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

Suppl. Fig. 4: Growth curves and waterfall plots of PDX models showing partial response (combination therapy only)

Each # indicates the last point in time one tumor was measured and taken out thereafter. Vertical gray bars indicate the end of the primary observational period of 28 days.Waterfall plots show the response after 59 days of treatment or before the end of the experiment compared with tumor volume at baseline. Each bar represents one tumor. Dotted lines indicate the cut-off values for progressive disease (PD) and partial response (PR). *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

Suppl. Fig. 5: Growth curves and waterfall plots of PDX models showing stable disease (combination therapy only)

Each # indicates the last point in time one tumor was measured and taken out thereafter. Vertical gray bars indicate the end of the primary observational period of 28 days.Waterfall plots show the response after 59 days of treatment or before the end of the experiment compared with tumor volume at baseline. Each bar represents one tumor. Dotted lines indicate the cut-off values for progressive disease (PD) and partial response (PR). *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

Suppl. Fig. 6: Growth curves and waterfall plots of PDX models showing progressive disease (combination therapy only)

Each # indicates the last point in time one tumor was measured and taken out afterwards. Vertical gray bars indicate end of the primary observational period of 28 days. Waterfall plots show the response after 59 days of treatment or before end of experiment compared with tumor volume at baseline. Each bar represents one tumor. Dotted lines indicate the cut-off values for progressive disease (PD) and partial response (PR). *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

Suppl. Figure 7: Additional tumors not shown in Fig. 3 receiving intermittent treatment

In tumors responding until day 59, treatment was paused until tumor volume reached at least 200mm³ again. In case of disease control upon retreatment for at least 30 days, the treatment was paused again. Treatment was re-initialized if the tumor regrew (200mm³ in case of partial response, 400mm³ in case of stable disease). The treatment pauses are highlighted in grey, treatment periods in blue. Only one tumor of the BoC137 PDX model showed secondary resistance already in the second treatment cycle (A, right panel, top growth curve).

Suppl. Fig. 8: Western Blots of the first set of PDX models tested

C, untreated control tumor; 5dCT, tumor harvested after combination therapy for 5 days.

Suppl. Fig. 9: Assessment of ERK and AKT phosphorylation in resistant and responding tumors

Phosphorylation of ERK and AKT in primarily resistant tumors (A), secondarily resistant tumors (B) and tumors with partial response (C). C, control tumor; 5dCT, tumor harvested after combination therapy for 5 days; CT, tumor harvested at the end of combination therapy. Most resistant tumors show an increase in ERK-phosphorylation at the end of treatment, suggesting pathway re-activation.

Suppl. Fig. 10: Whole exome sequencing results

BoC51, BoC109, BoC117, and BoC122 were primary resistant to vertical inhibition with cetuximab and trametinib (suffix CT). Secondary resistant tumors BoC2 and BoC56 were compared to control tumors (suffix K).

Suppl. Table 1

PDX	gene	variant	amino.acid
BoC2	APC	stop_gained	p.Q1367Ter
BoC2	HNF1A	frameshift_variant	p.P291QfsTer51
BoC2	KRAS	missense_variant	p.G12D
BoC2	STK11	frameshift_variant	p.P281RfsTer6
BoC9	APC	stop_gained	p.Q1378Ter
BoC9	HNF1A	frameshift_variant	p.P291QfsTer51
BoC9	KRAS	missense_variant	p.G12D
BoC9	TP53	missense_variant	p.R175H
BoC14	APC	stop_gained	p.R876Ter
BoC14	FBXW7	missense_variant	p.R399Q
BoC14	KRAS	missense_variant	p.A146T
BoC14	MET	missense_variant	p.T1010I
BoC14	PTEN	stop_gained	p.Q17Ter
BoC19	CTNNB1	missense_variant	p.S45F
BoC19	KRAS	missense_variant	p.A146T
BoC19	TP53	missense_variant	p.R175H
BoC46	FBXW7	missense_variant	p.R399Q
BoC46	KRAS	missense_variant	p.G13D
BoC46	TP53	missense_variant	p.R175H
BoC47	KRAS	missense_variant	p.G12D
BoC47	TP53	missense_variant	p.C141Y
BoC51	KRAS	missense_variant	p.G12D
BoC56	APC	stop_gained	p.Q1429Ter
BoC56	HNF1A	frameshift_variant	p.P291QfsTer51
BoC56	KRAS	missense_variant	p.G12C
BoC64	KRAS	missense_variant	p.G12D
BoC64	VHL	missense_variant	p.S111G
BoC78	KRAS	missense_variant	p.G12D
BoC78	TP53	missense_variant	p.P72R
BoC80	KRAS	missense_variant	p.G12V
BoC105	KRAS	missense_variant	p.G12V
BoC105	PIK3CA	missense_variant	p.E545K

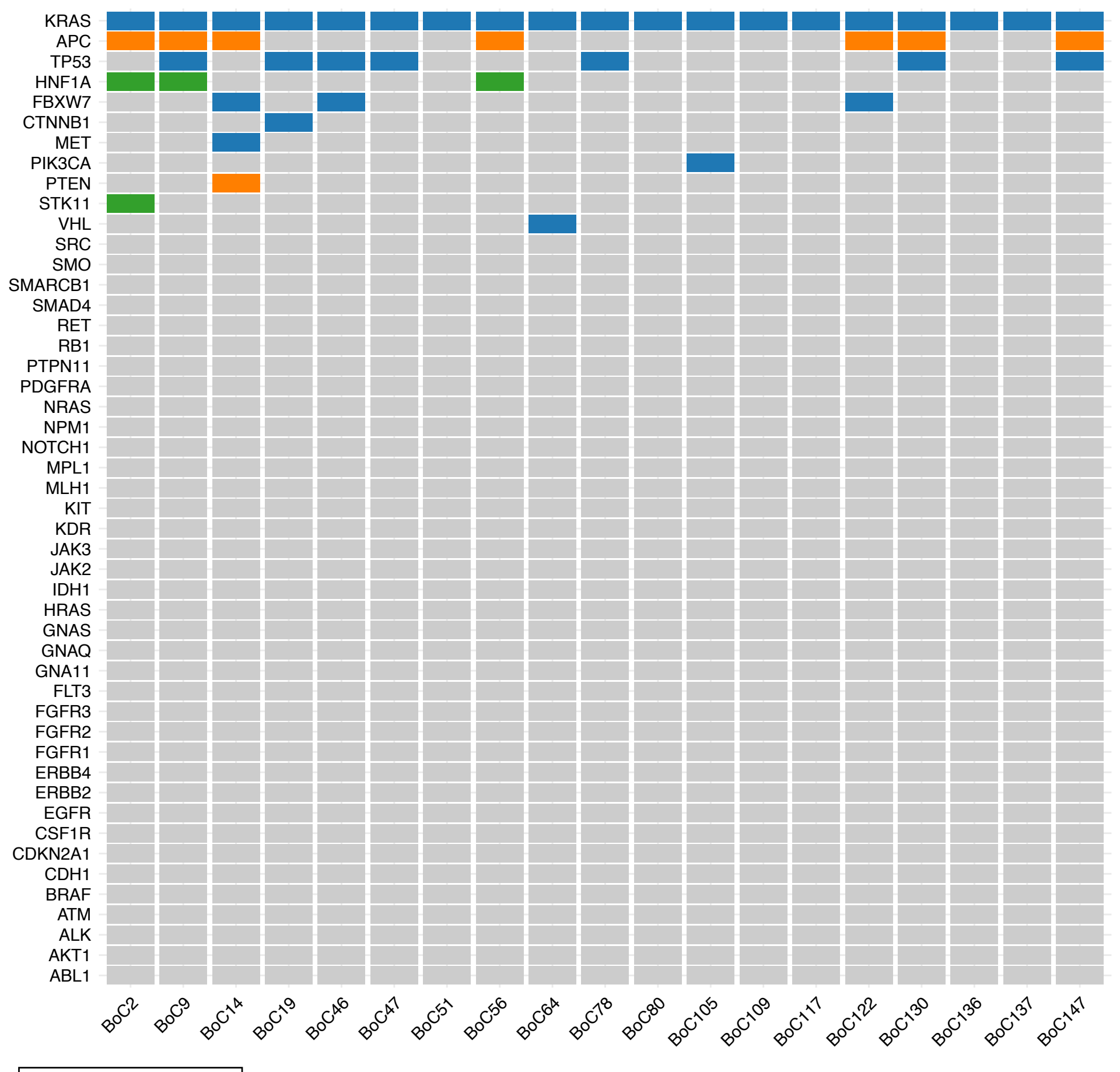
BoC109	KRAS	missense_variant	p.G13D
BoC117	KRAS	missense_variant	p.G12D
BoC122	APC	stop_gained	p.E1379Ter
BoC122	FBXW7	missense_variant	p.R505C
BoC122	KRAS	missense_variant	p.G12D
BoC130	APC	stop_gained	p.Q1367Ter
BoC130	KRAS	missense_variant	p.G12D
BoC130	TP53	missense_variant	p.R273H
BoC136	KRAS	missense_variant	p.G12V
BoC137	KRAS	missense_variant	p.G12D
BoC147	APC	stop_gained	p.R1114Ter
BoC147	KRAS	missense_variant	p.G12A
BoC147	TP53	missense_variant	p.R248Q

Suppl. Table 2

pdx_new2	response	gene	variant_type	protein_letter
BoC122_CT5	primary resistant	FBXW7	missense	p.R505C
BoC122_CT5	primary resistant	APC	stop_gained	p.E1379Ter
BoC122_CT5	primary resistant	KRAS	missense	p.G12D
BoC117_CT7	primary resistant	KRAS	missense	p.G12D
BoC109_CT2	primary resistant	CDC73	stop_gained	p.R9Ter
BoC109_CT2	primary resistant	KRAS	missense	p.G13D
BoC109_CT2	primary resistant	NF1	stop_gained	p.R826Ter
BoC51_CT5	primary resistant	KRAS	missense	p.G12D
BoC2_K4	untreated contr.	APC	stop_gained	p.Q1367Ter
BoC2_K4	untreated contr.	KRAS	missense	p.G12D
BoC2_CT6	secondary resistant	APC	stop_gained	p.Q1367Ter
BoC2_CT6	secondary resistant	KRAS	missense	p.G12D
BoC56_K1	untreated contr.	ACVR1	missense	p.G328V
BoC56_K1	untreated contr.	APC	stop_gained	p.Q1429Ter
BoC56_K1	untreated contr.	KRAS	missense	p.G12C
BoC56 CT4	secondary resistant	ACVR1	missense	p.G328V
BoC56 CT4	secondary resistant	APC	stop_gained	p.Q1429Ter
BoC56 CT4	secondary resistant	KRAS	missense	p.G12C

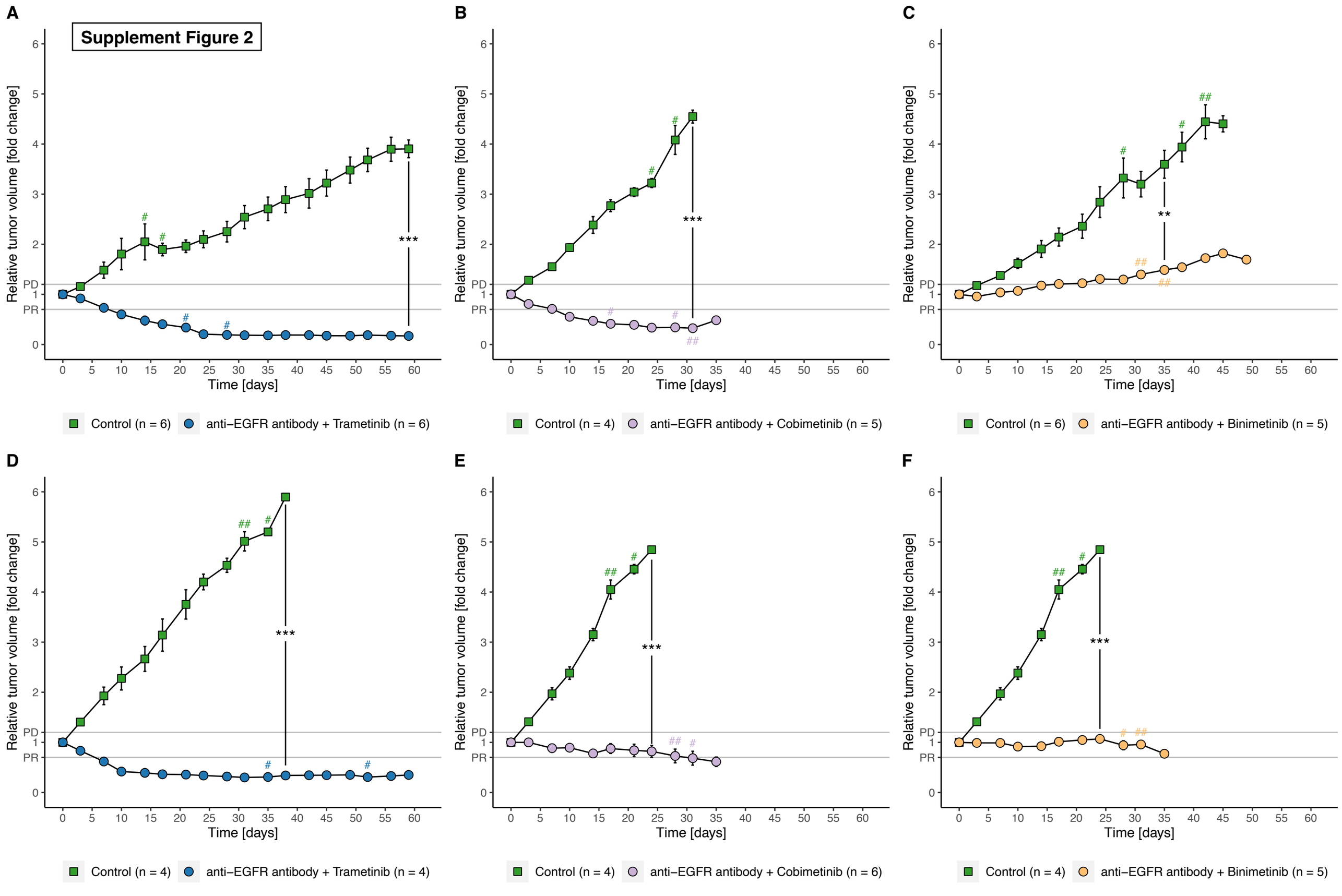
Supplement Table 4

Xenograft Model	overall response	prediction	d.CMS1	d.CMS2	d.CMS3	d.CMS4	p.value	FDR
BoC109_K5	primary resistant	CMS1	0.631601353	0.788431338	0.784025432	0.669195662	0.001	0.0018125
BoC137_K5	partial response	CMS1	0.669194477	0.681091723	0.678559053	0.710930112	0.007992008	0.012528013
BoC2_K3	secondary resistant	CMS1	0.640473134	0.713492363	0.699673808	0.723930893	0.001	0.0018125
BoC64_K1	primary resistant	CMS1	0.632896202	0.728554032	0.657721579	0.782731538	0.001	0.0018125
BoC117_K7	primary resistant	CMS2	0.777720818	0.648861808	0.711894881	0.808805662	0.001	0.0018125
BoC47_K7	partial response	CMS2	0.797619402	0.656023251	0.744769548	0.826778781	0.008991009	0.013723119
BoC51_K1	primary resistant	CMS3	0.628891996	0.79452842	0.593918342	0.669839805	0.001	0.0018125
BoC56_K1	secondary resistant	CMS3	0.727319498	0.6687746	0.616951422	0.632473103	0.001	0.0018125
BoC122_K3	primary resistant	CMS4	0.668496017	0.725570096	0.691137298	0.626319497	0.001	0.0018125



Supplement Figure 1

Supplement Figure 2



Supplement Figure 3

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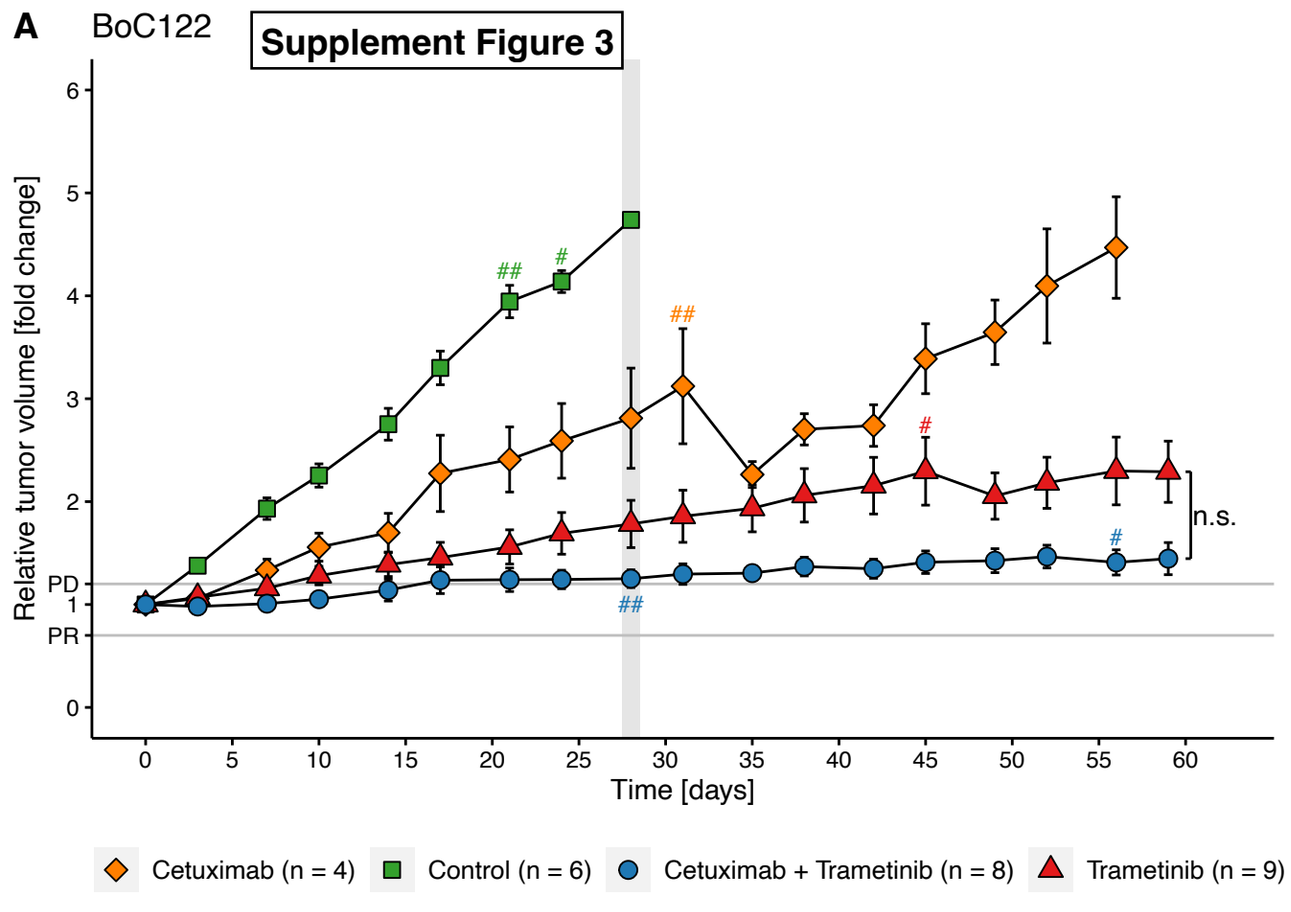

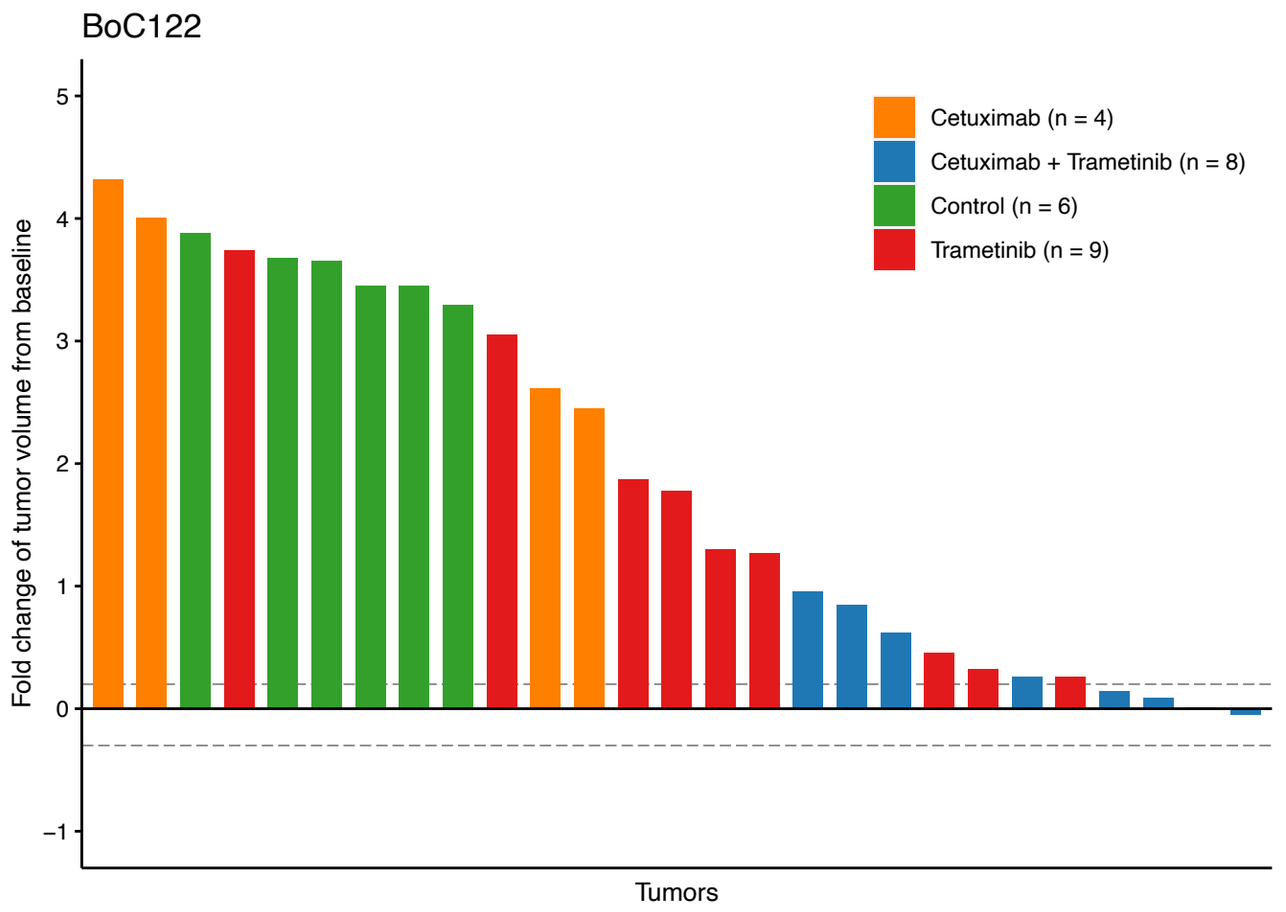

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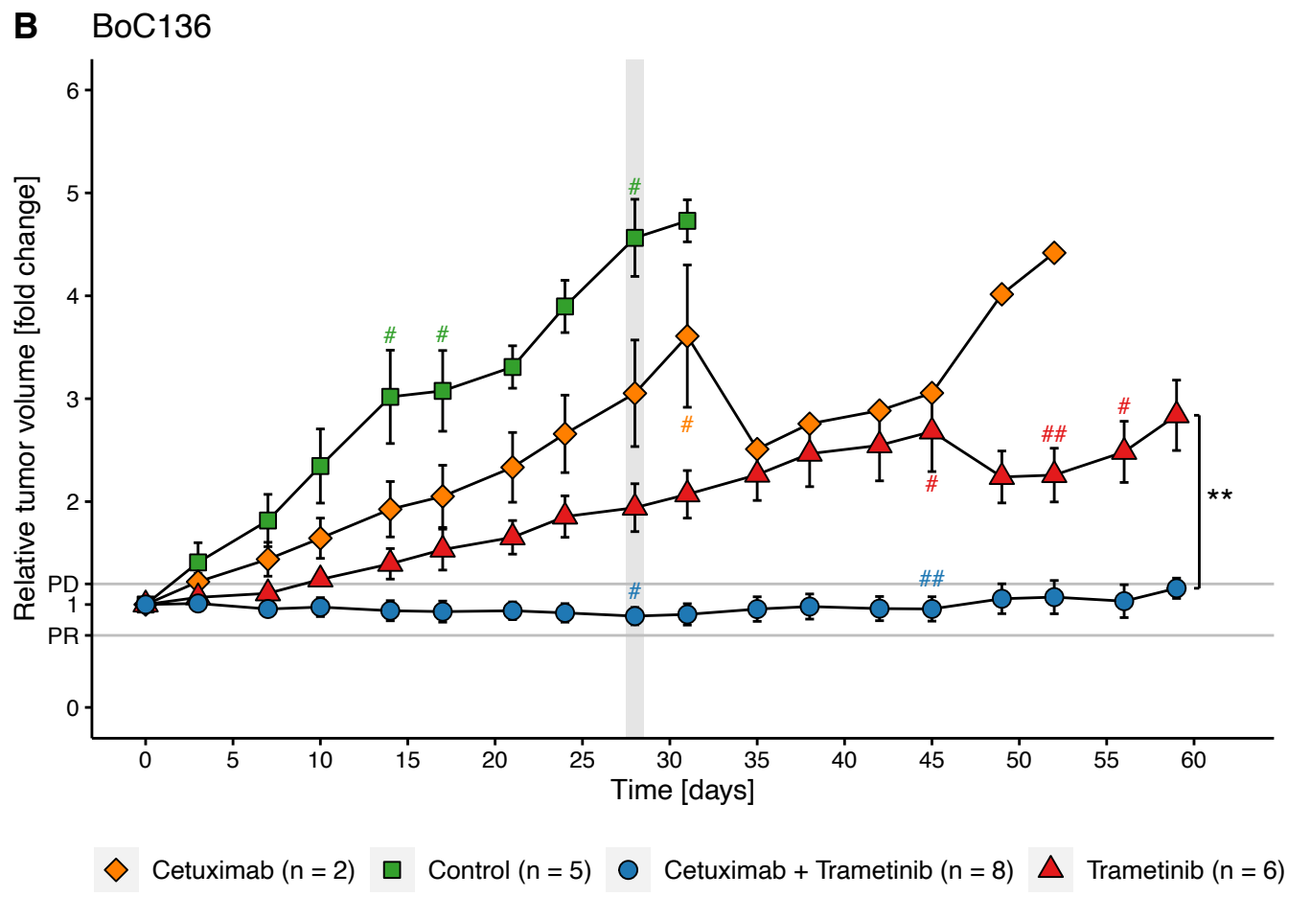

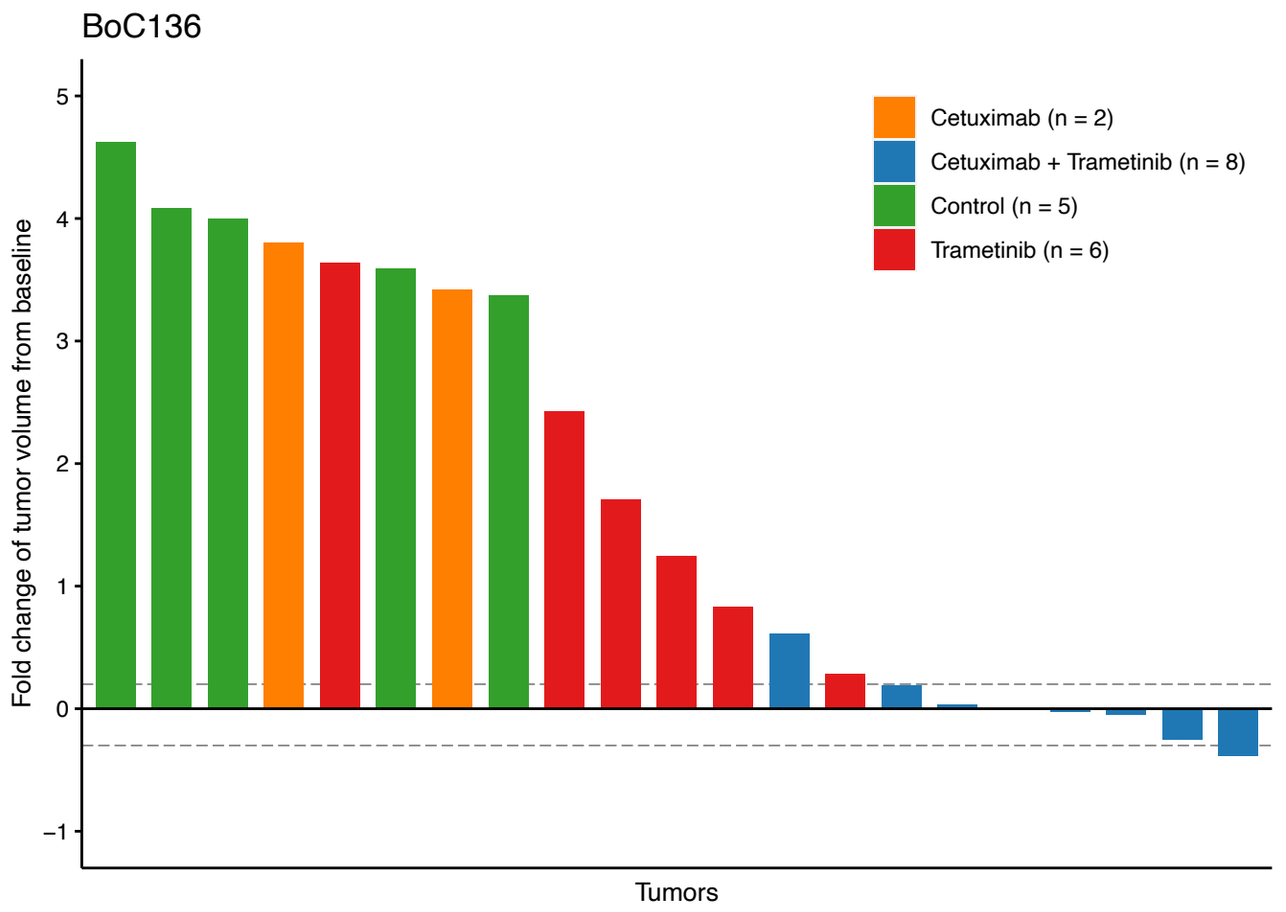

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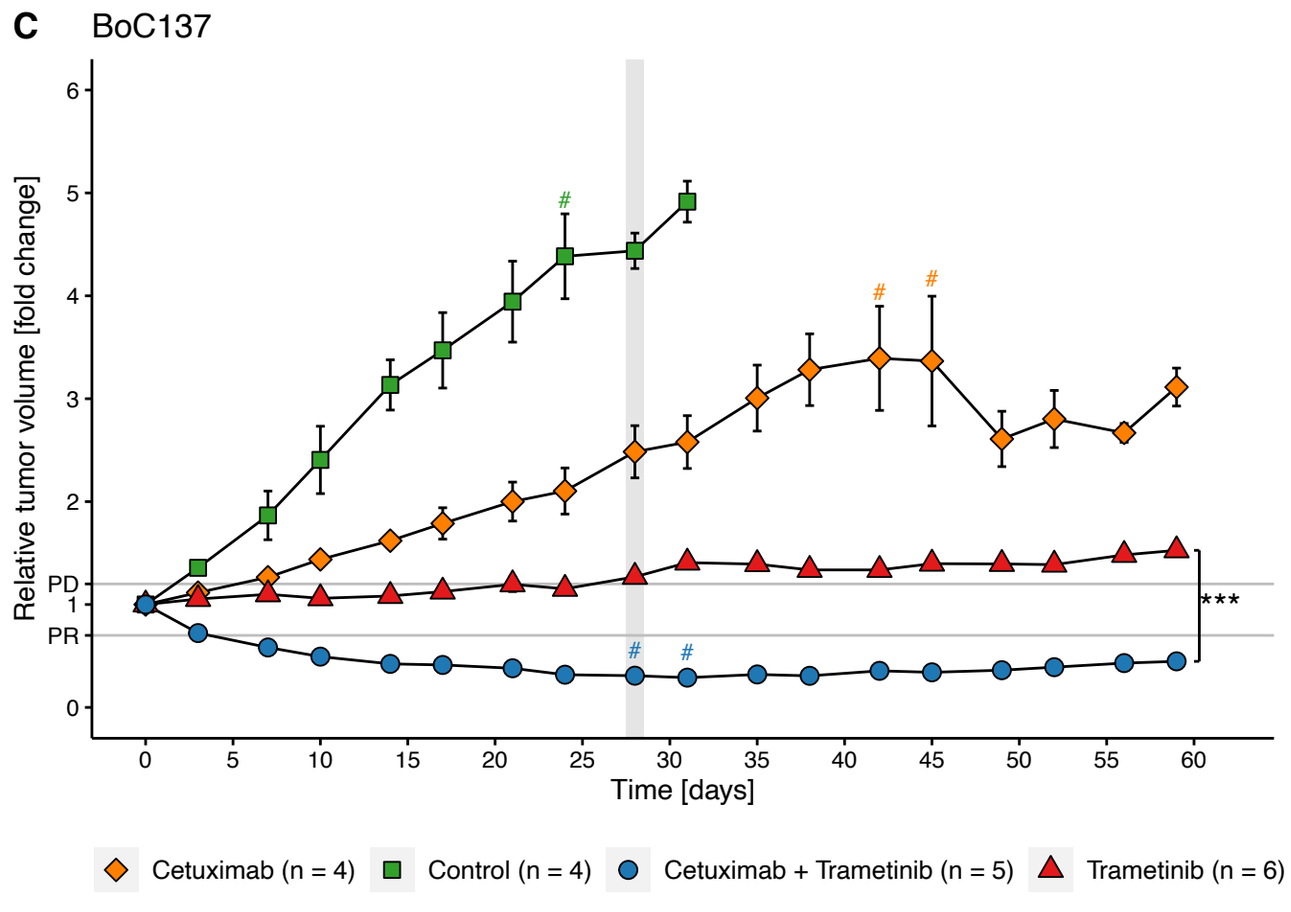

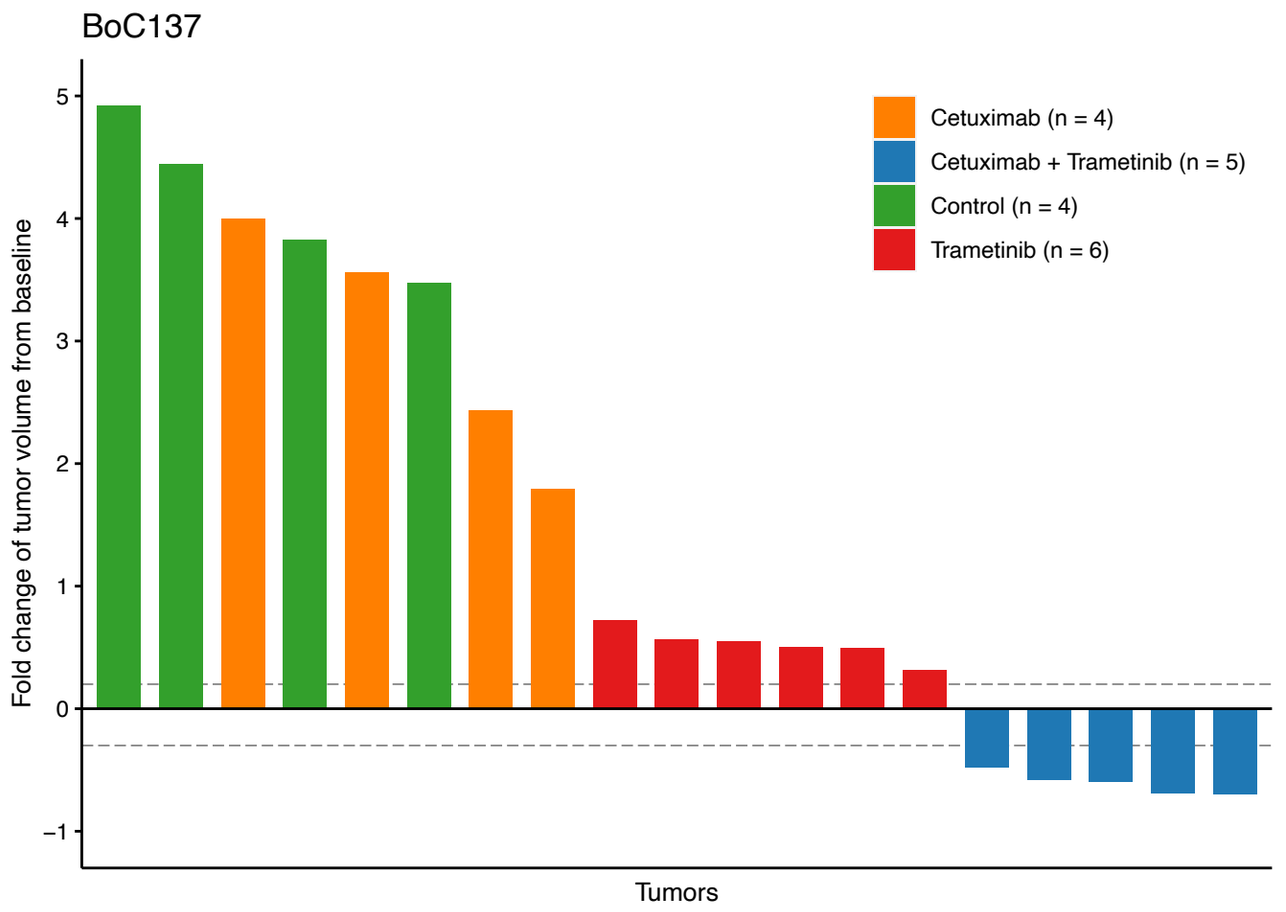

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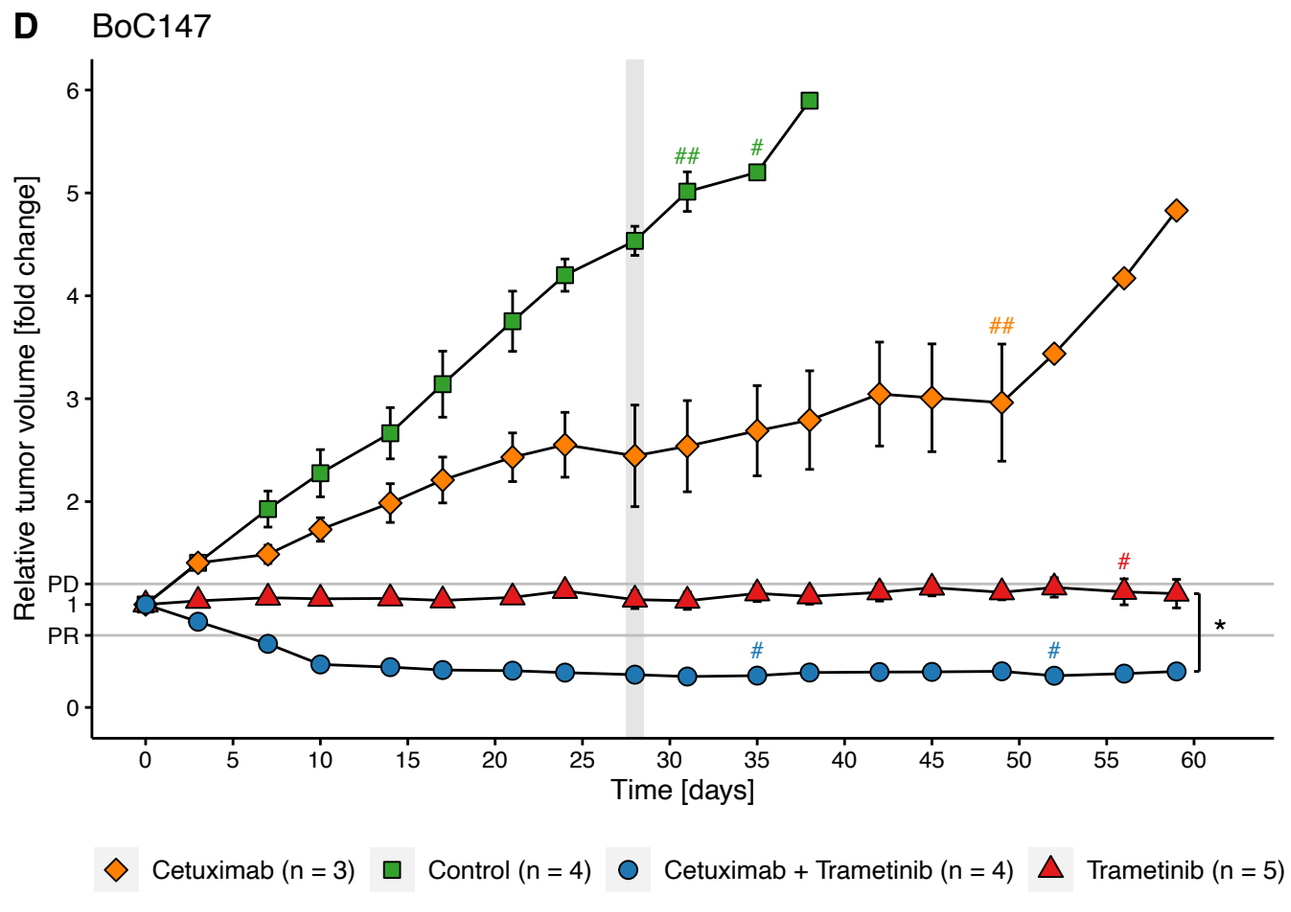
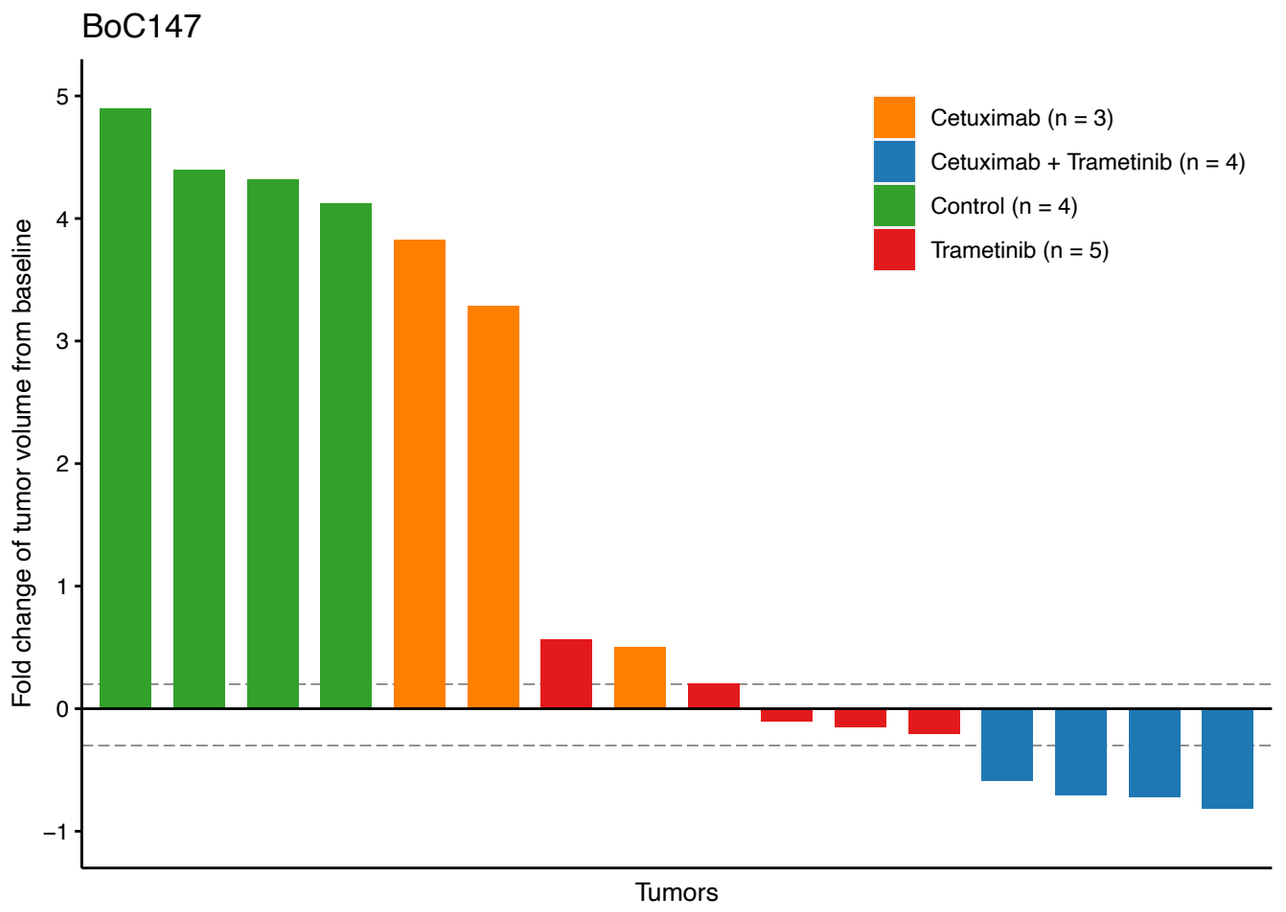
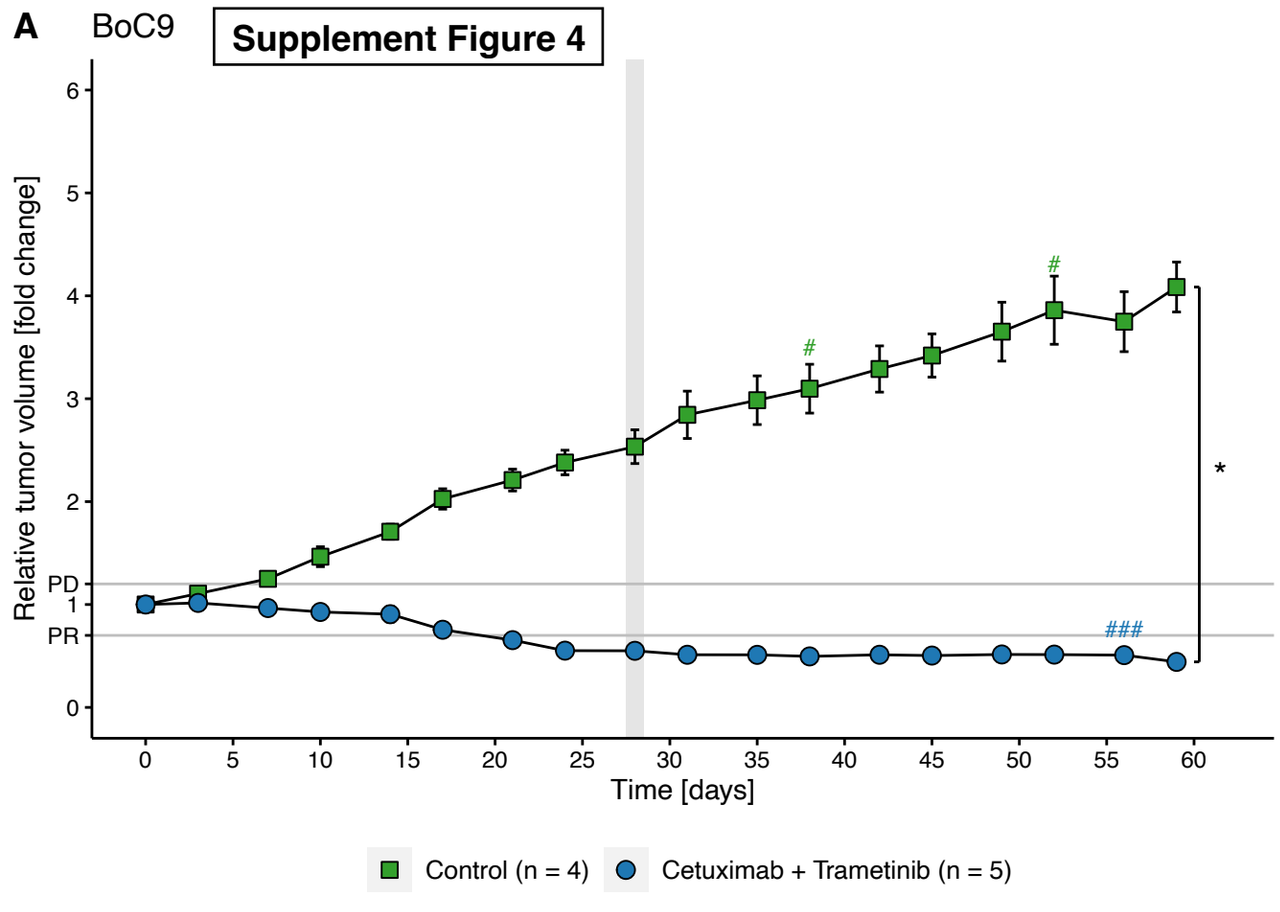
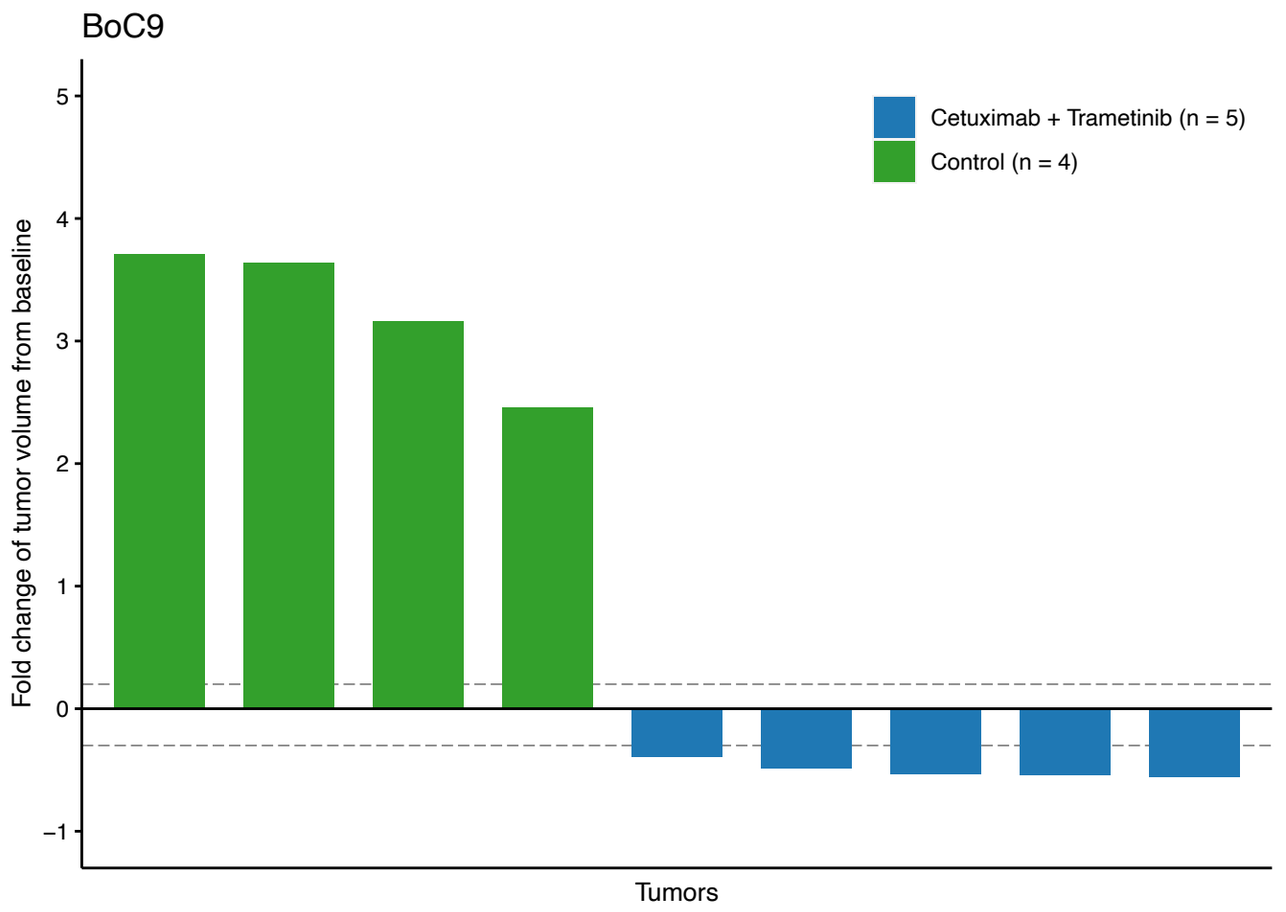




Supplement Figure 4

A BoC9





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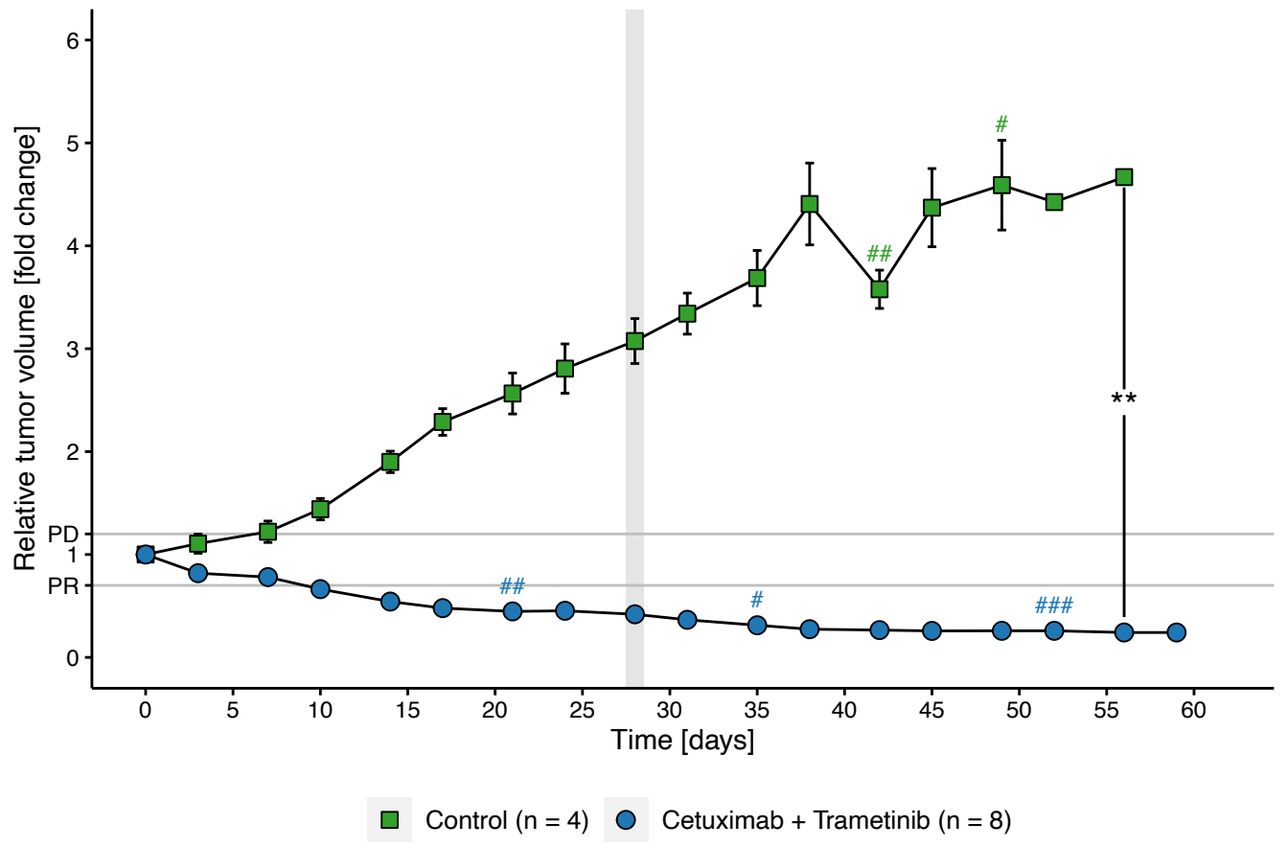

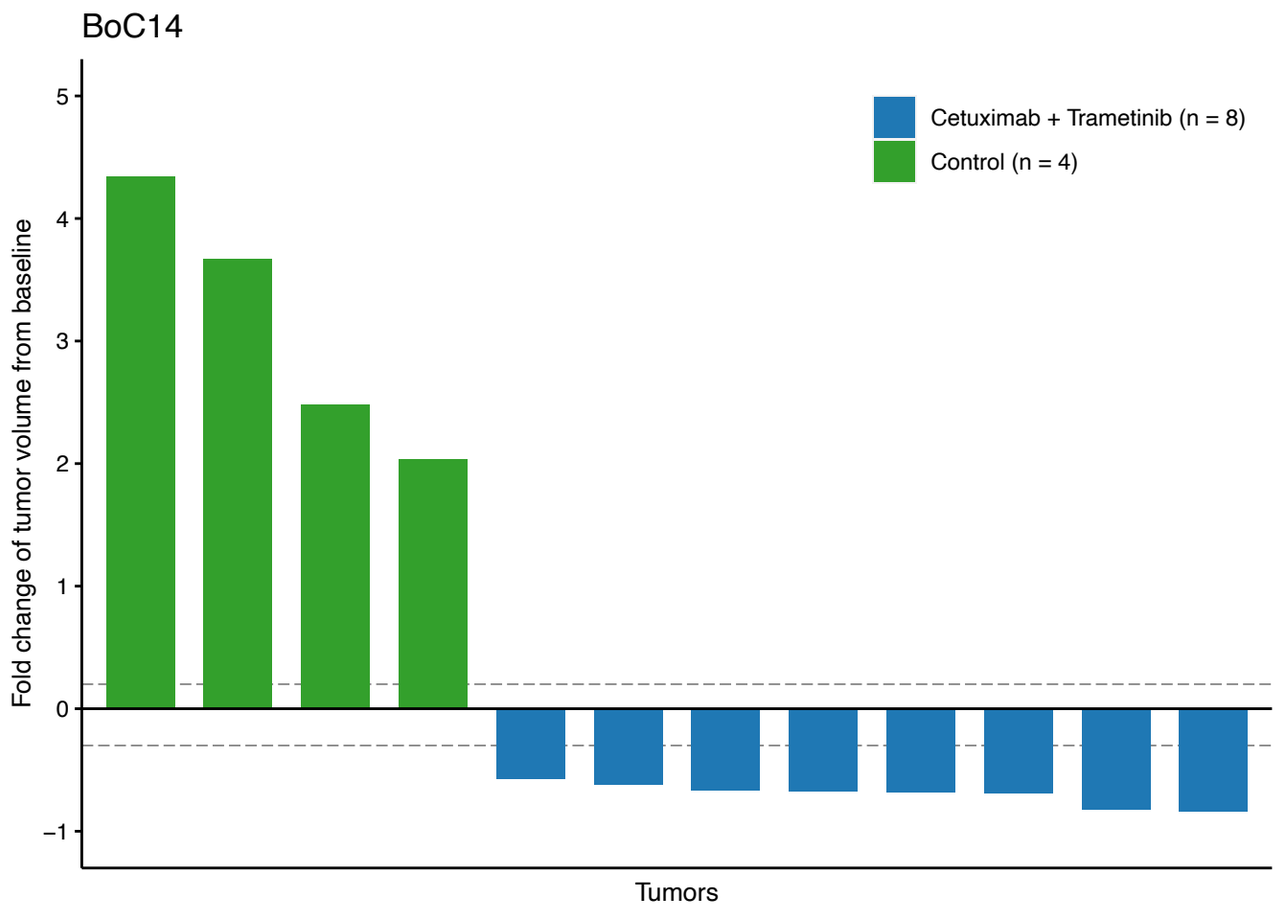

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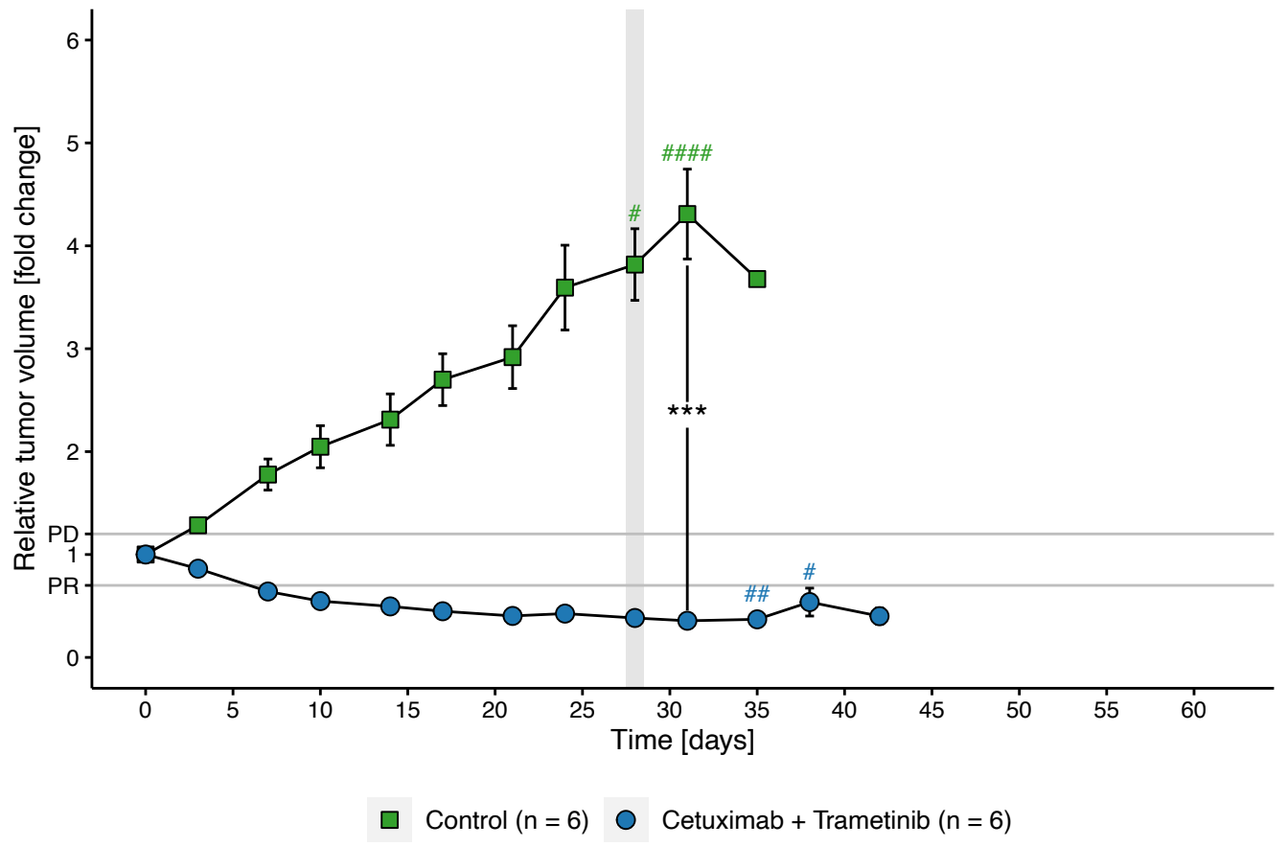

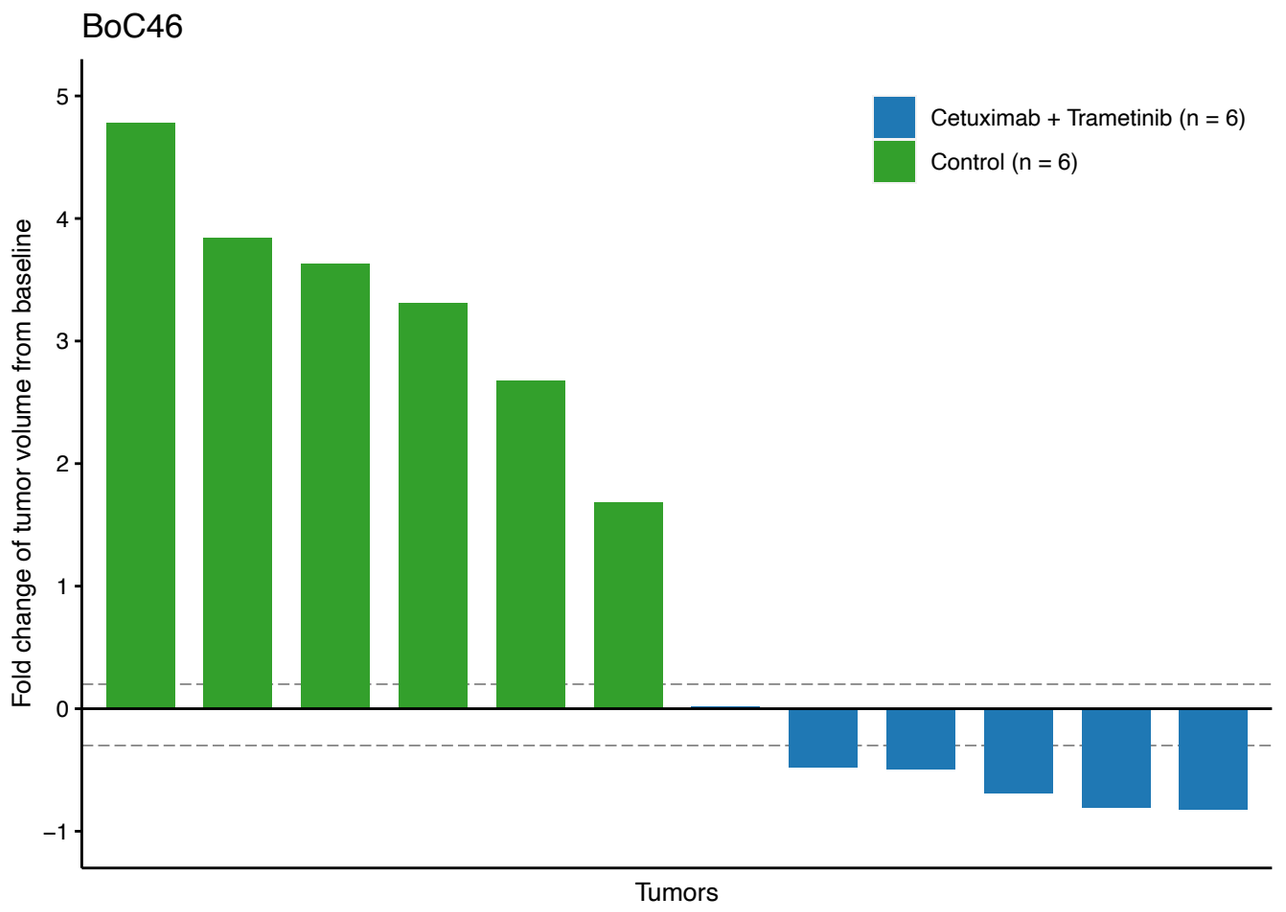

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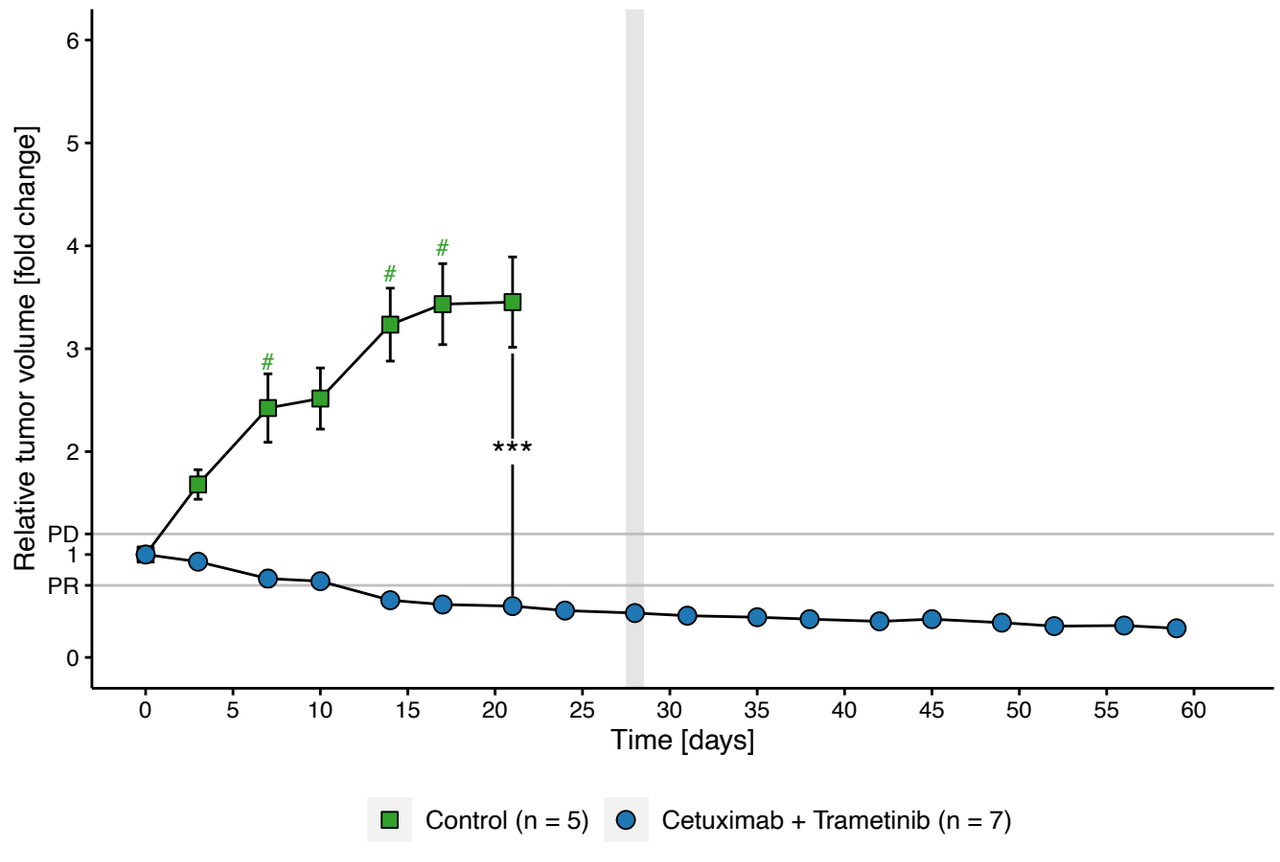

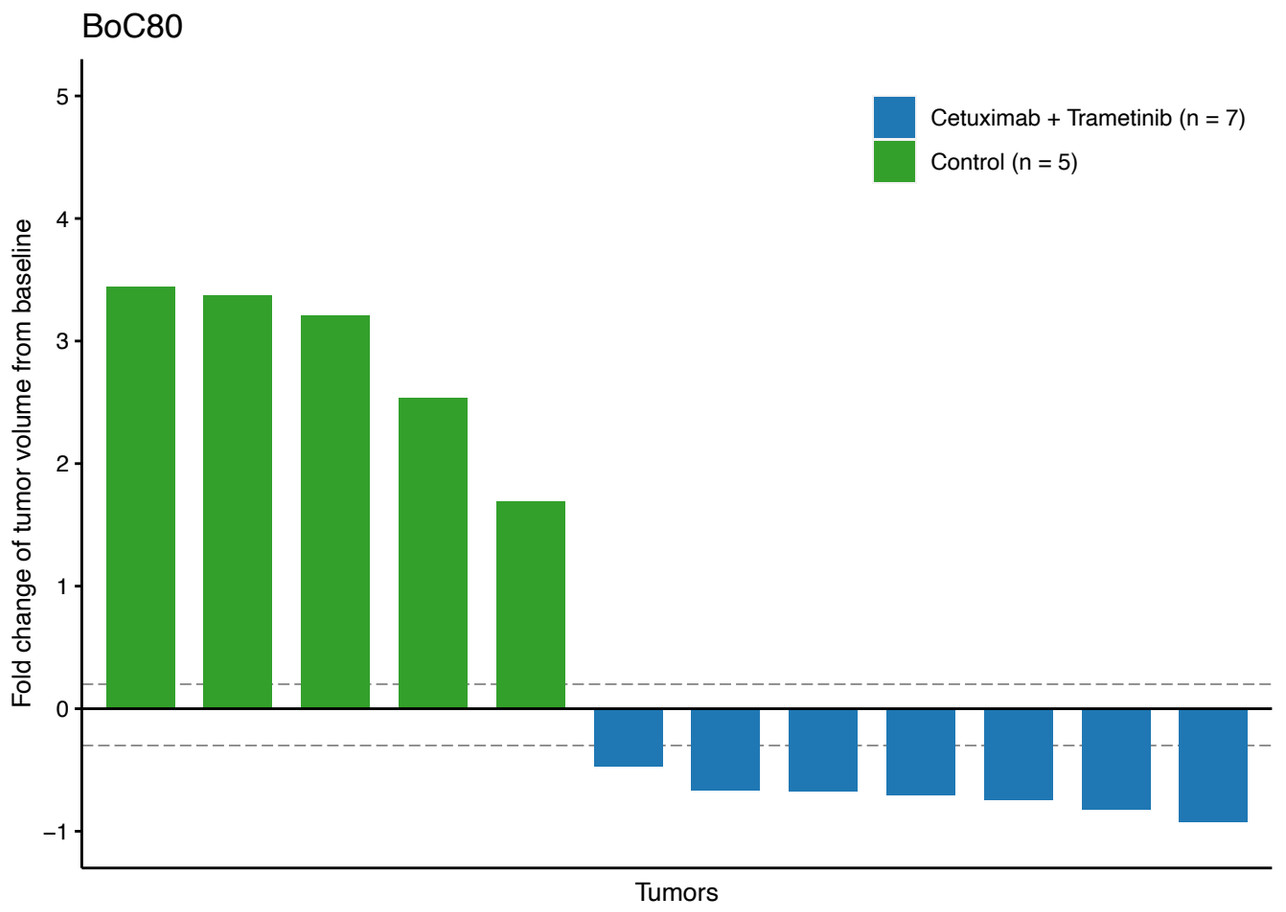

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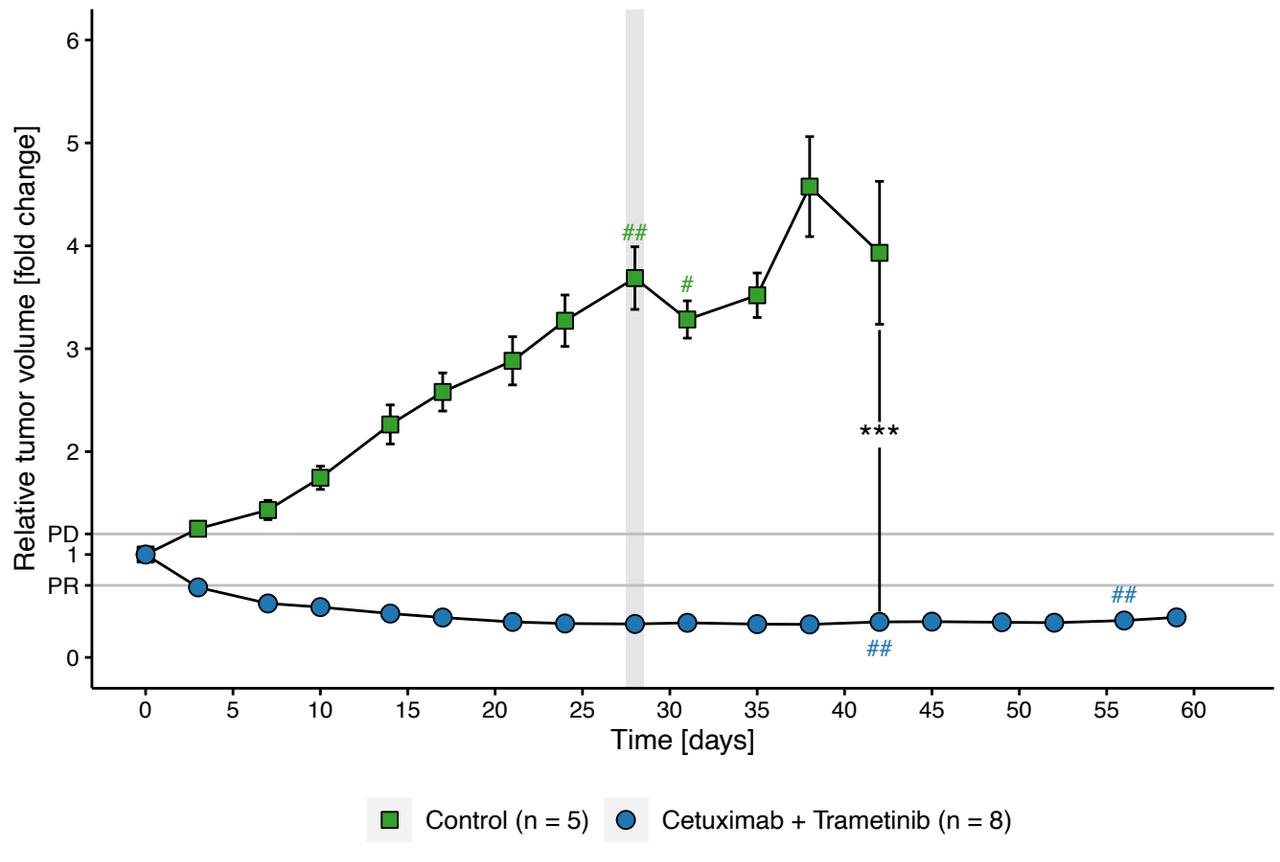
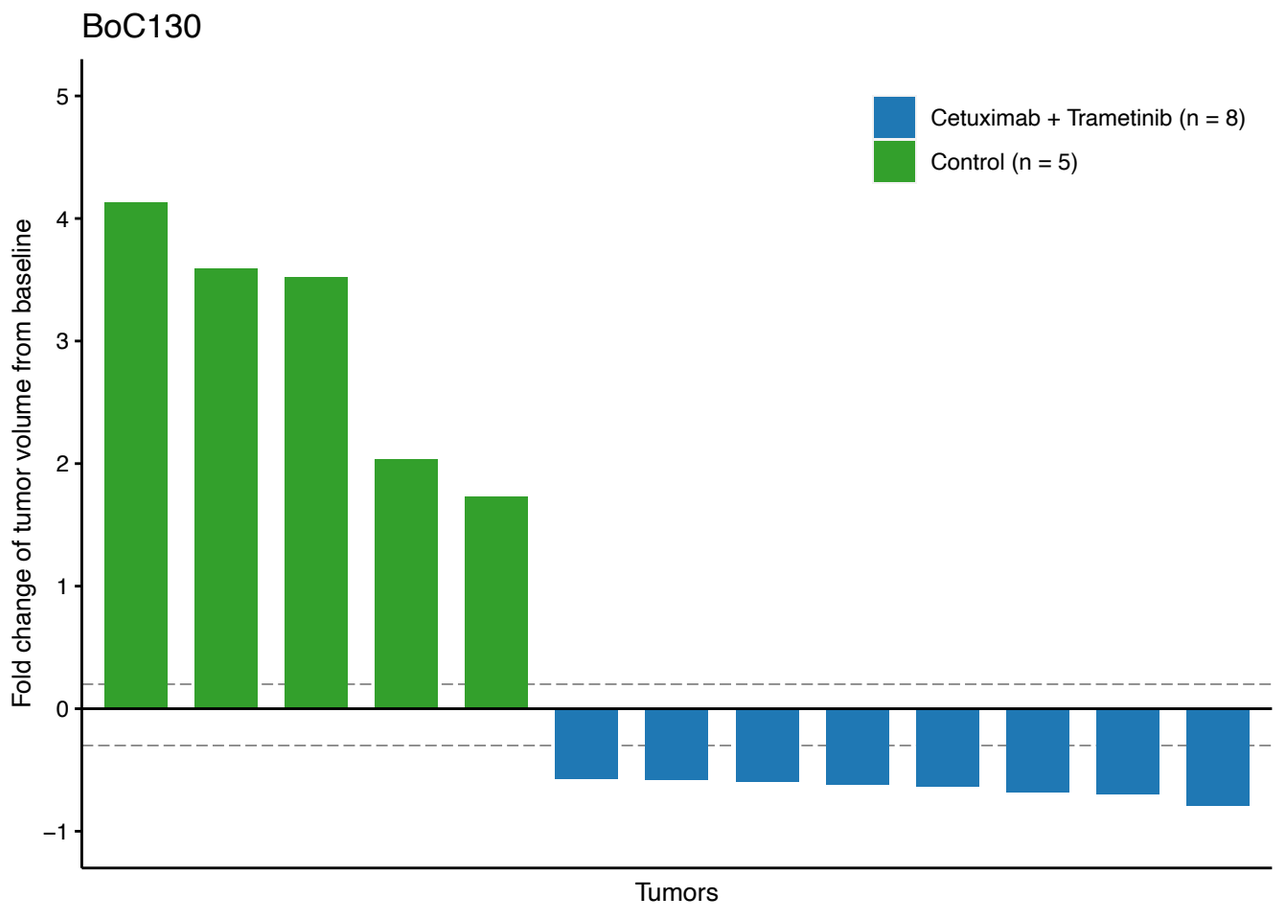
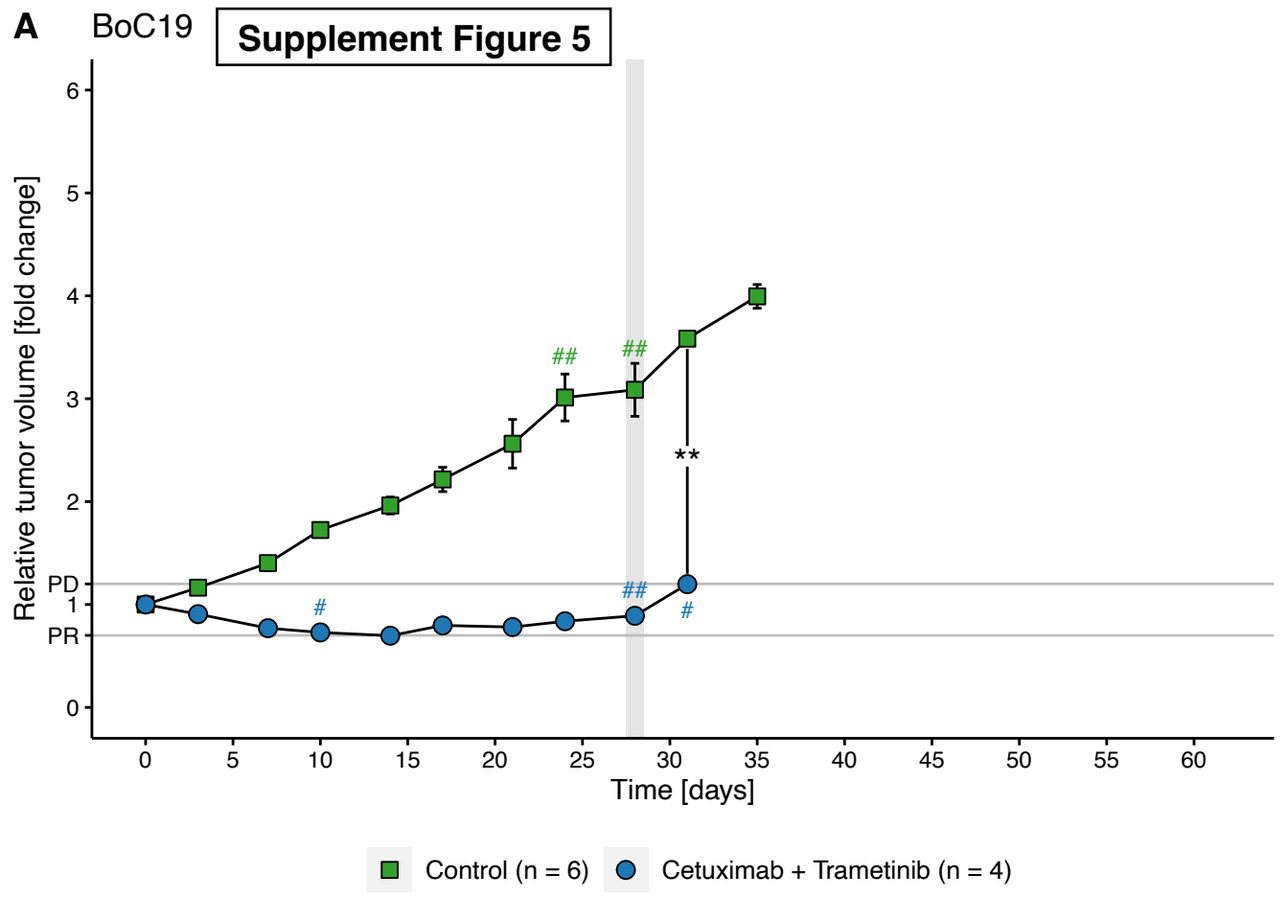
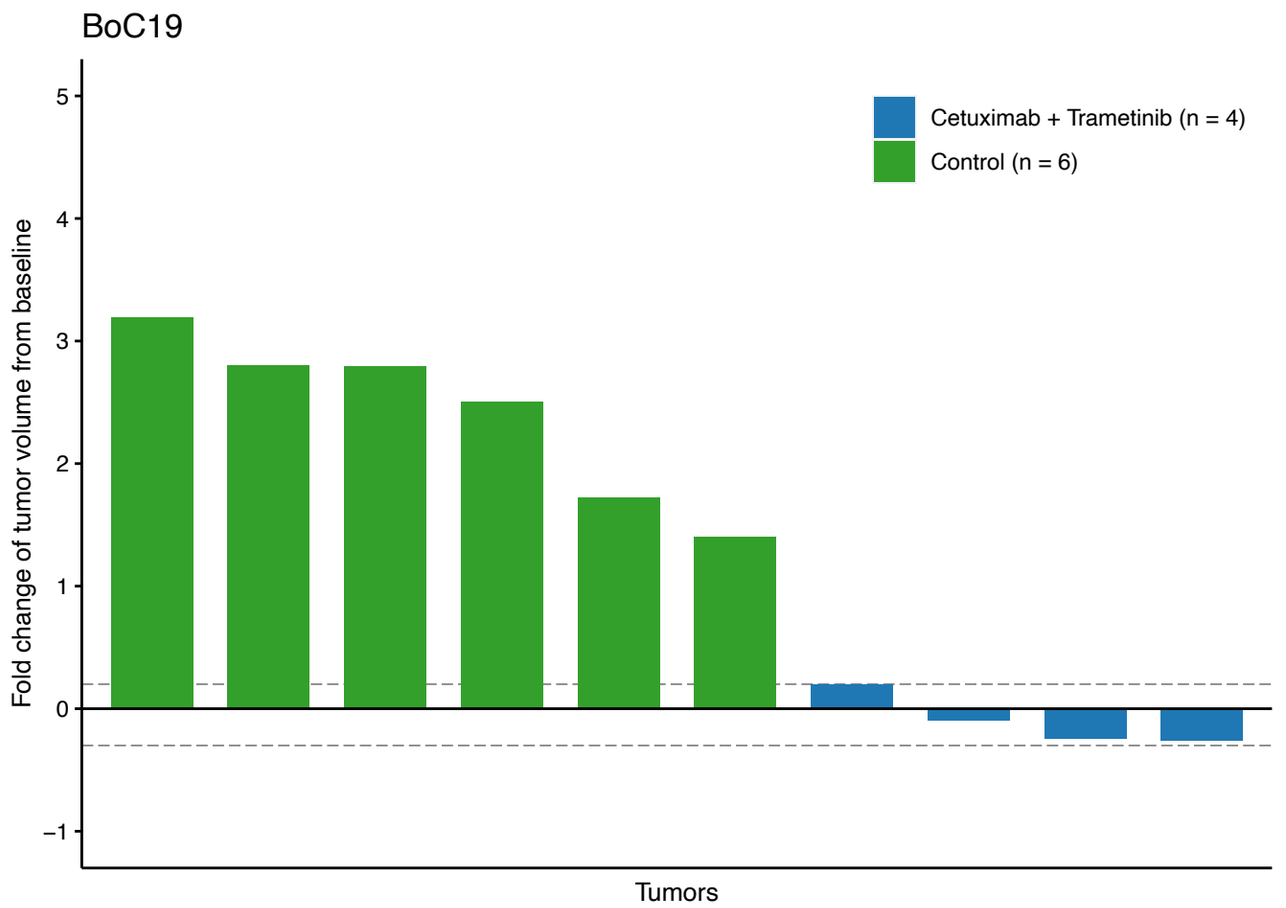
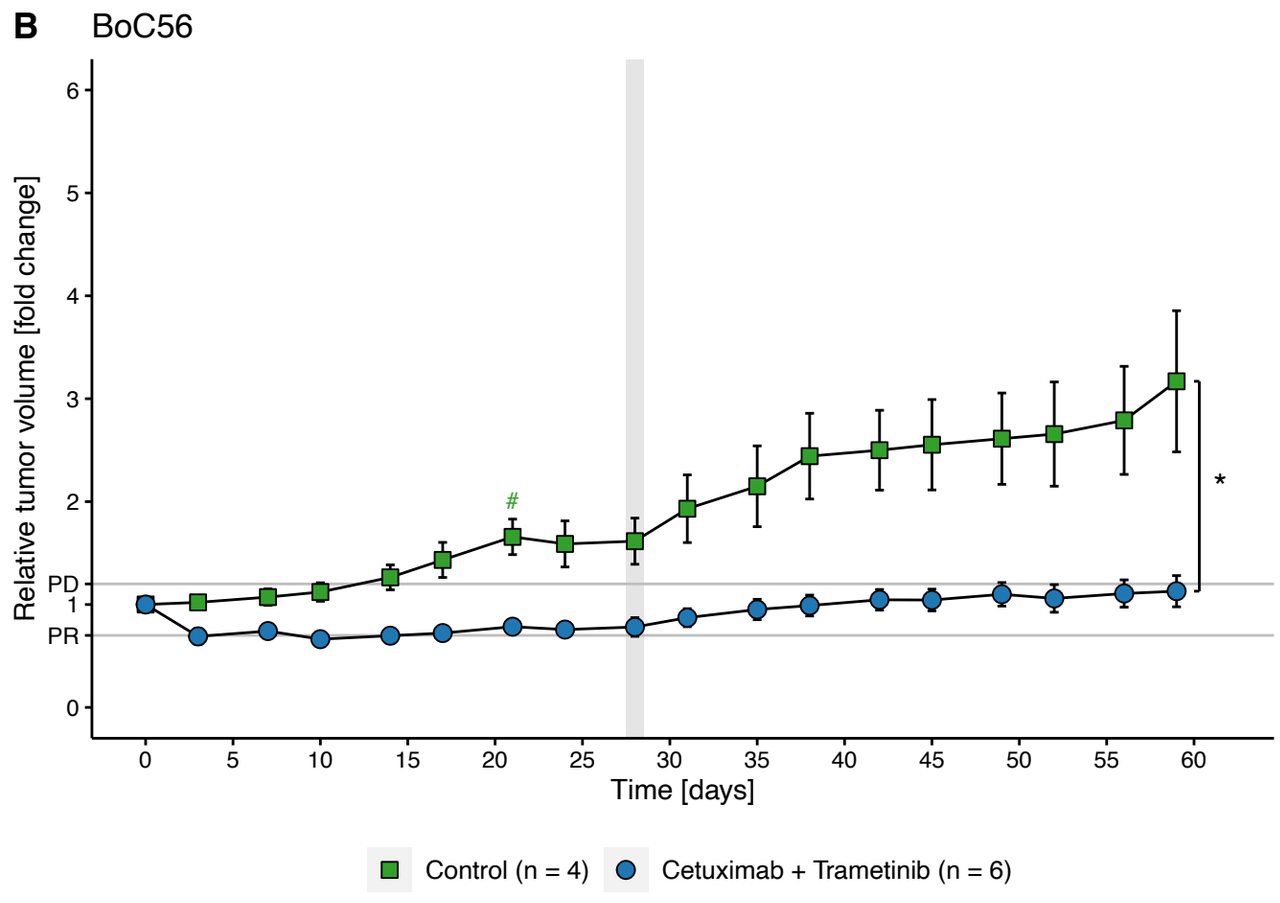


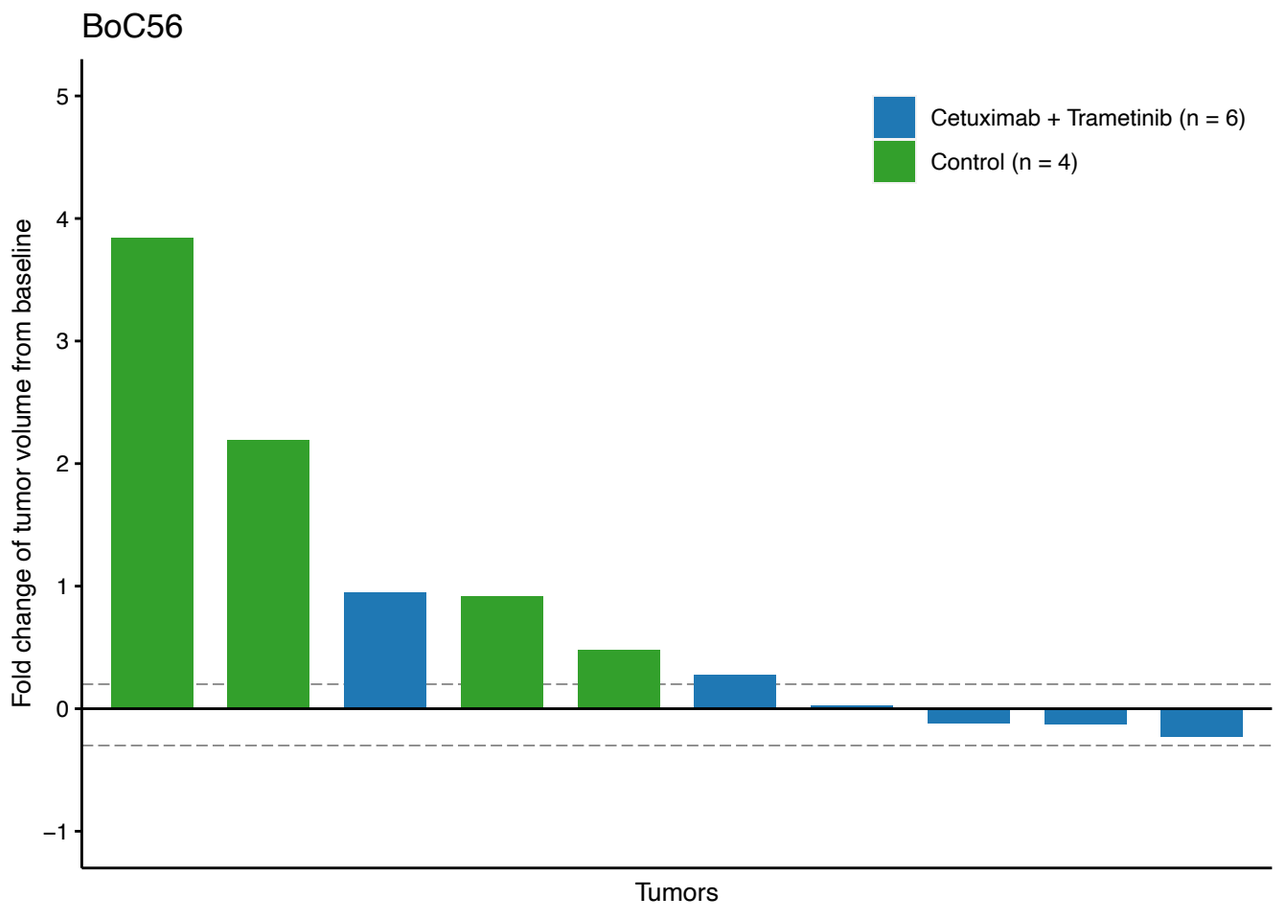


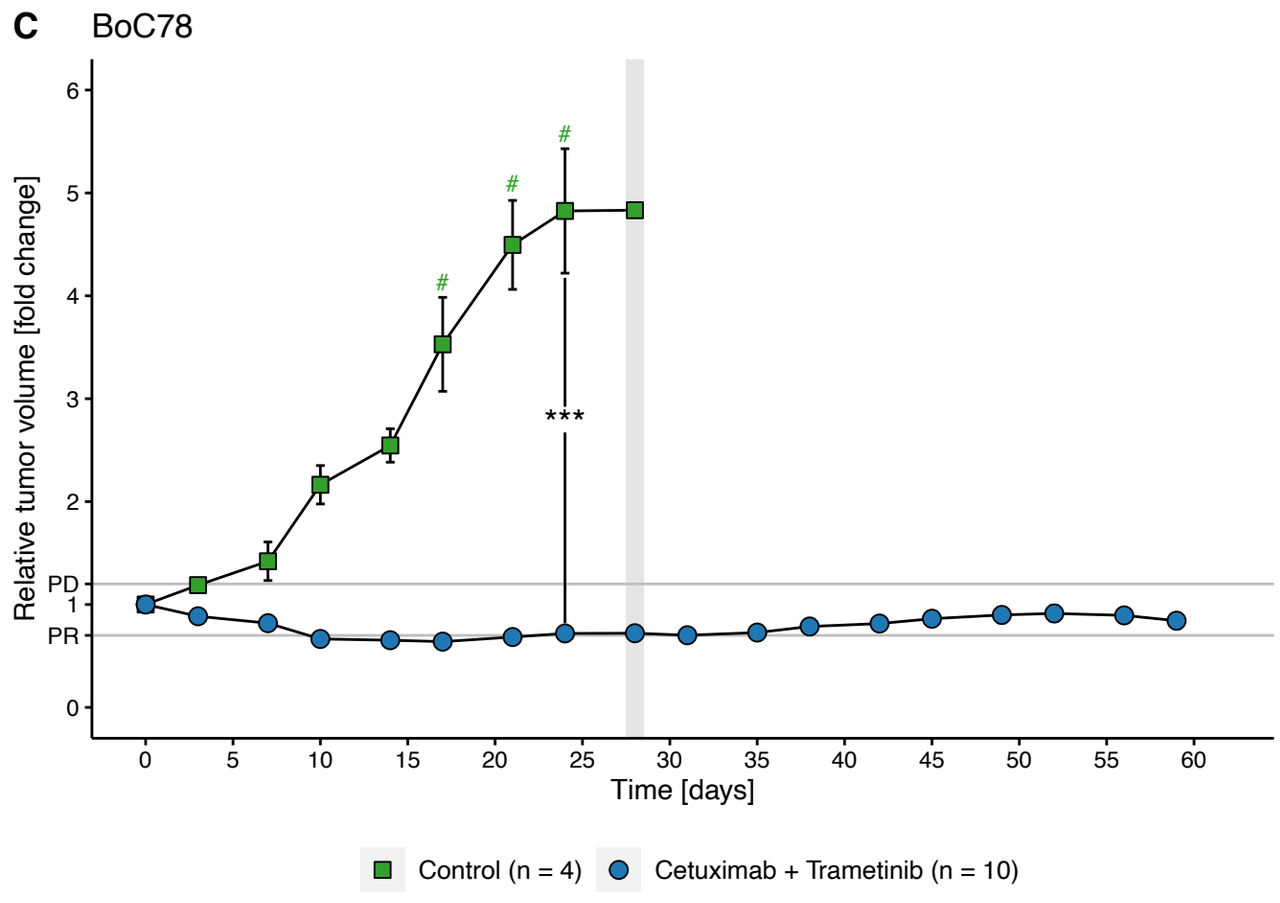
Supplement Figure 5

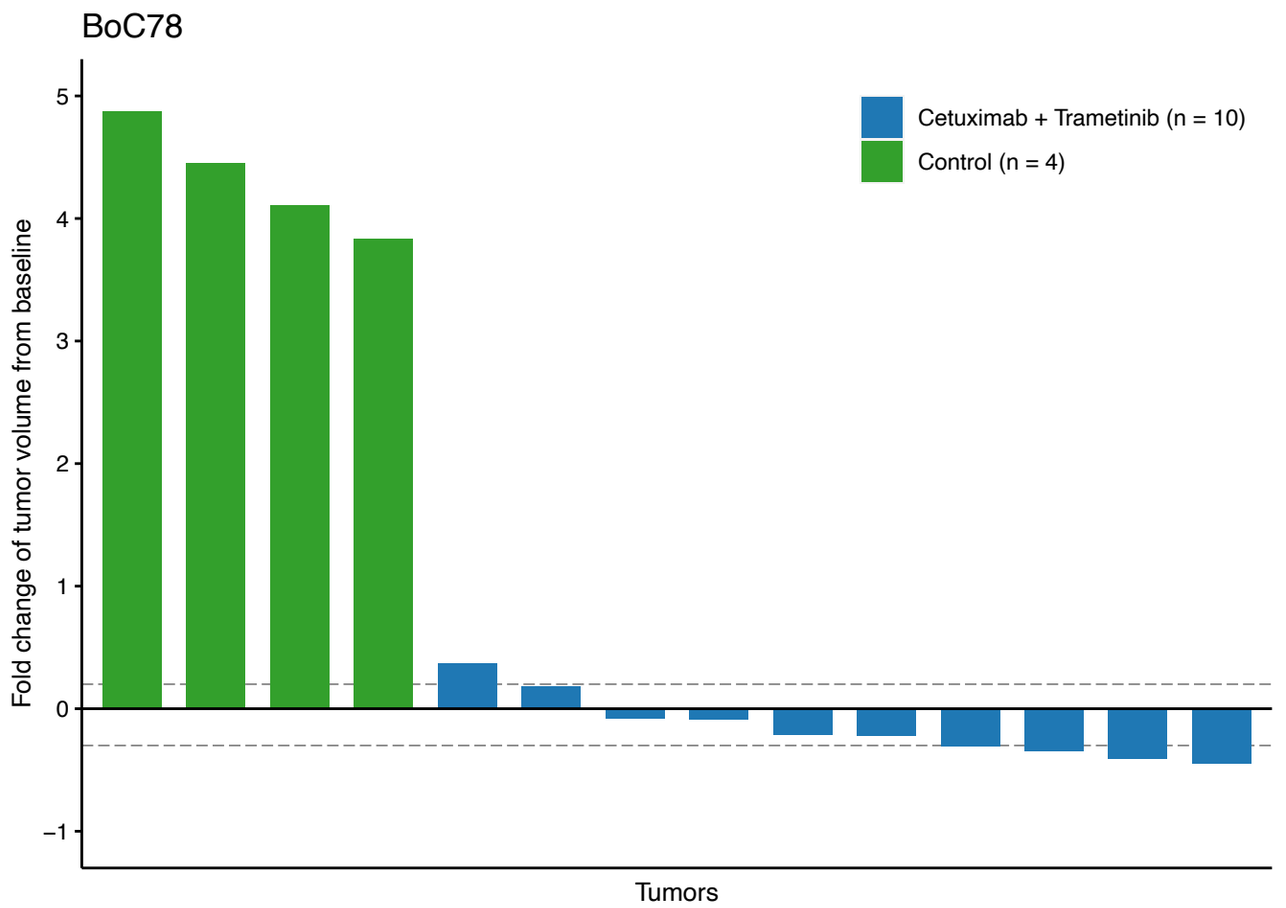












Supplement Figure 6

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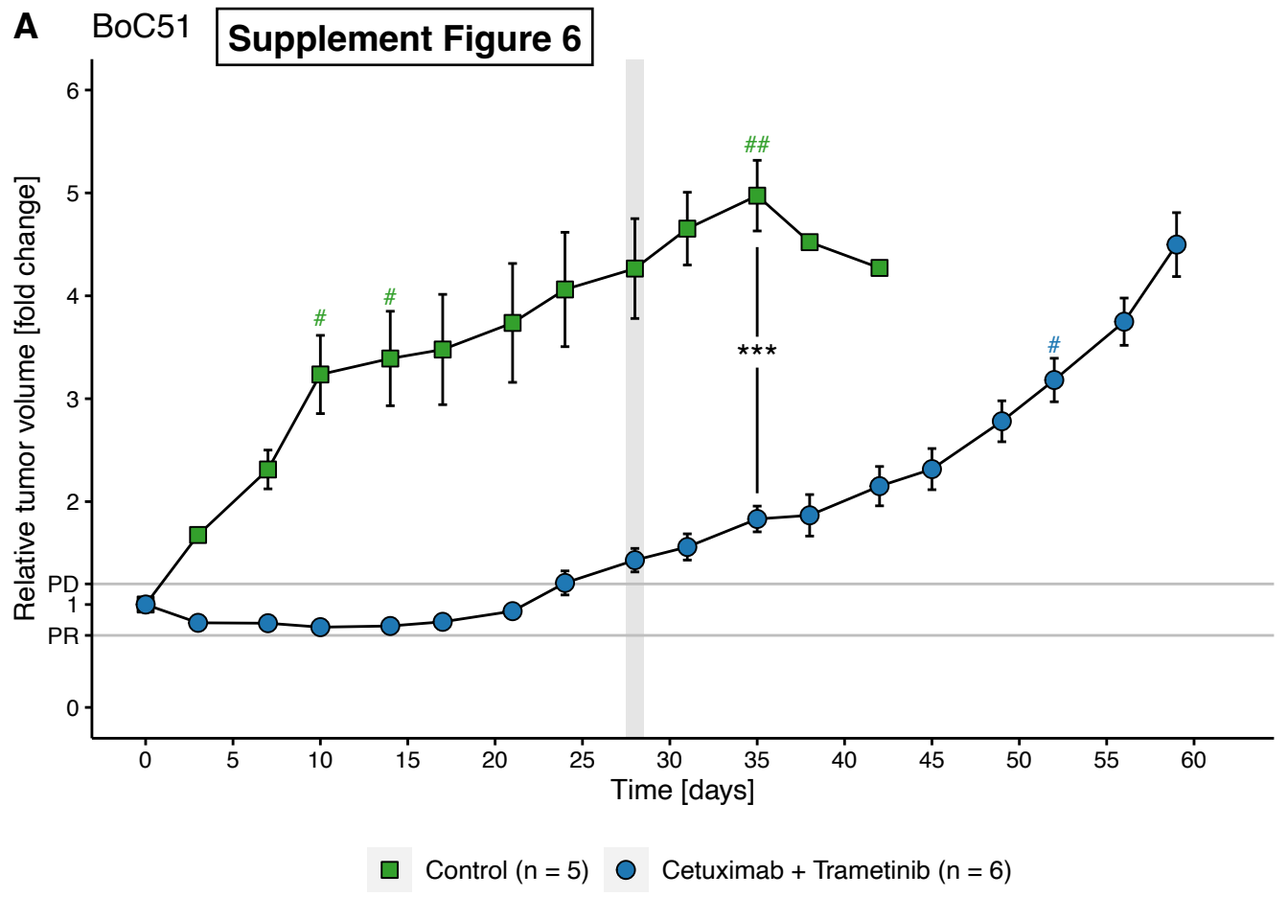

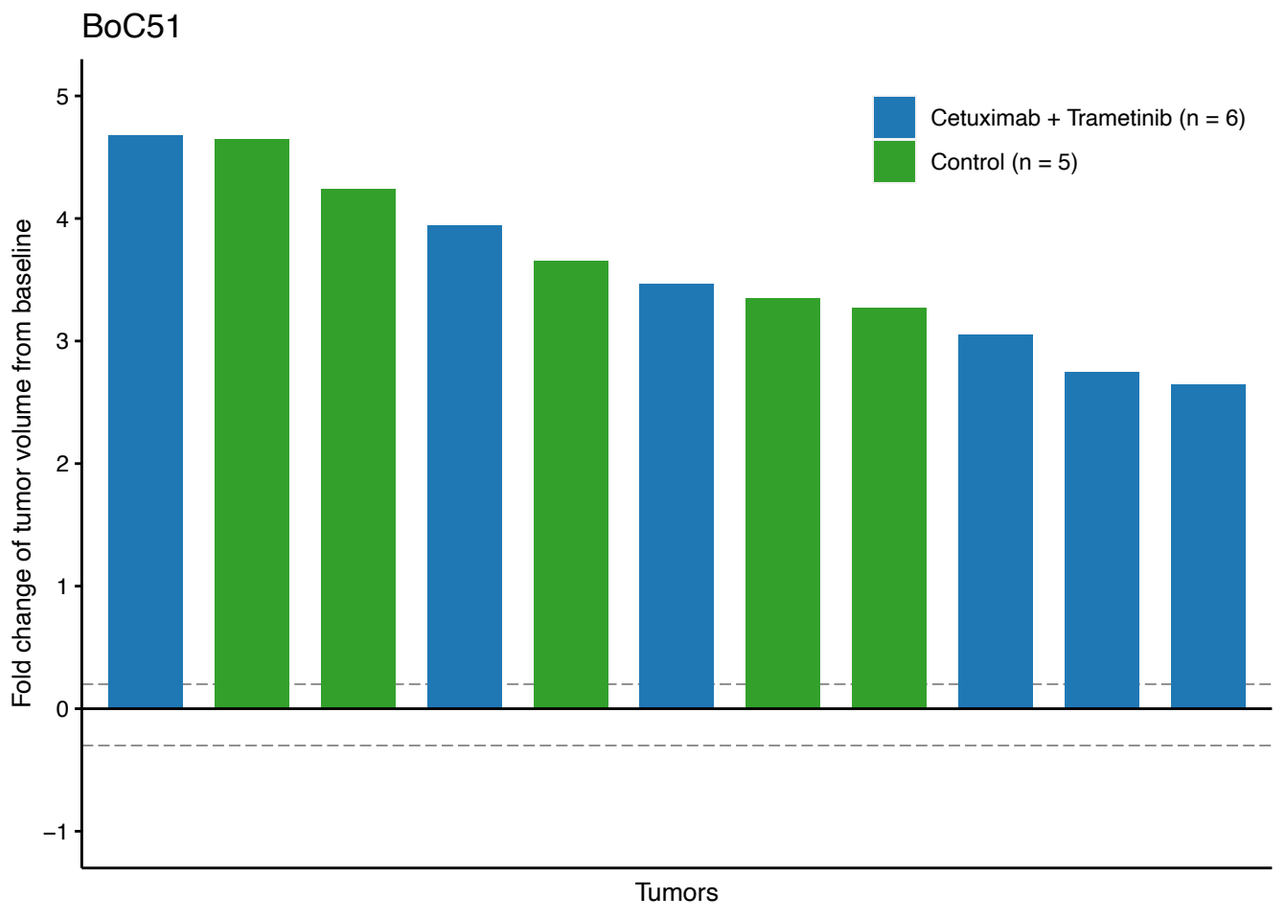

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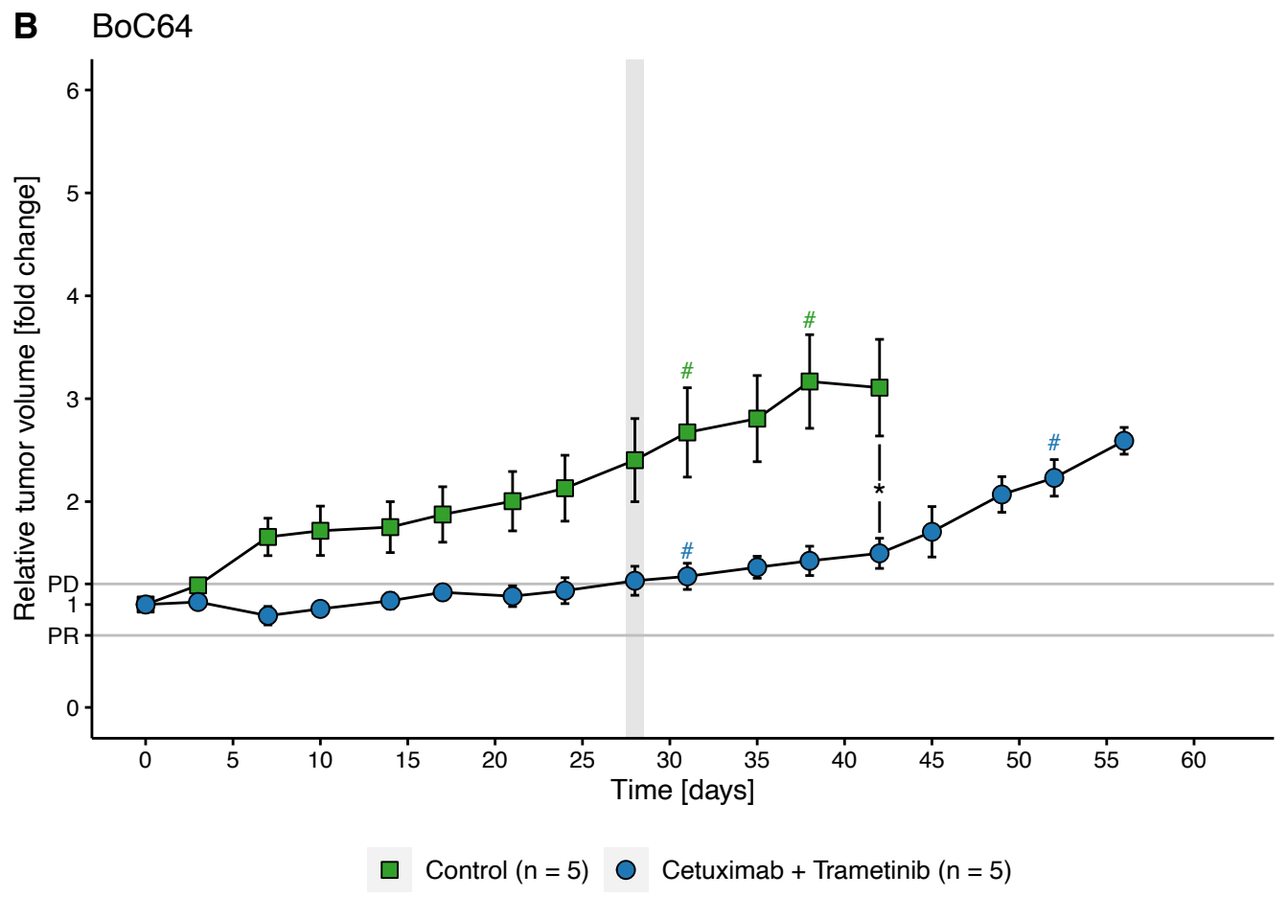

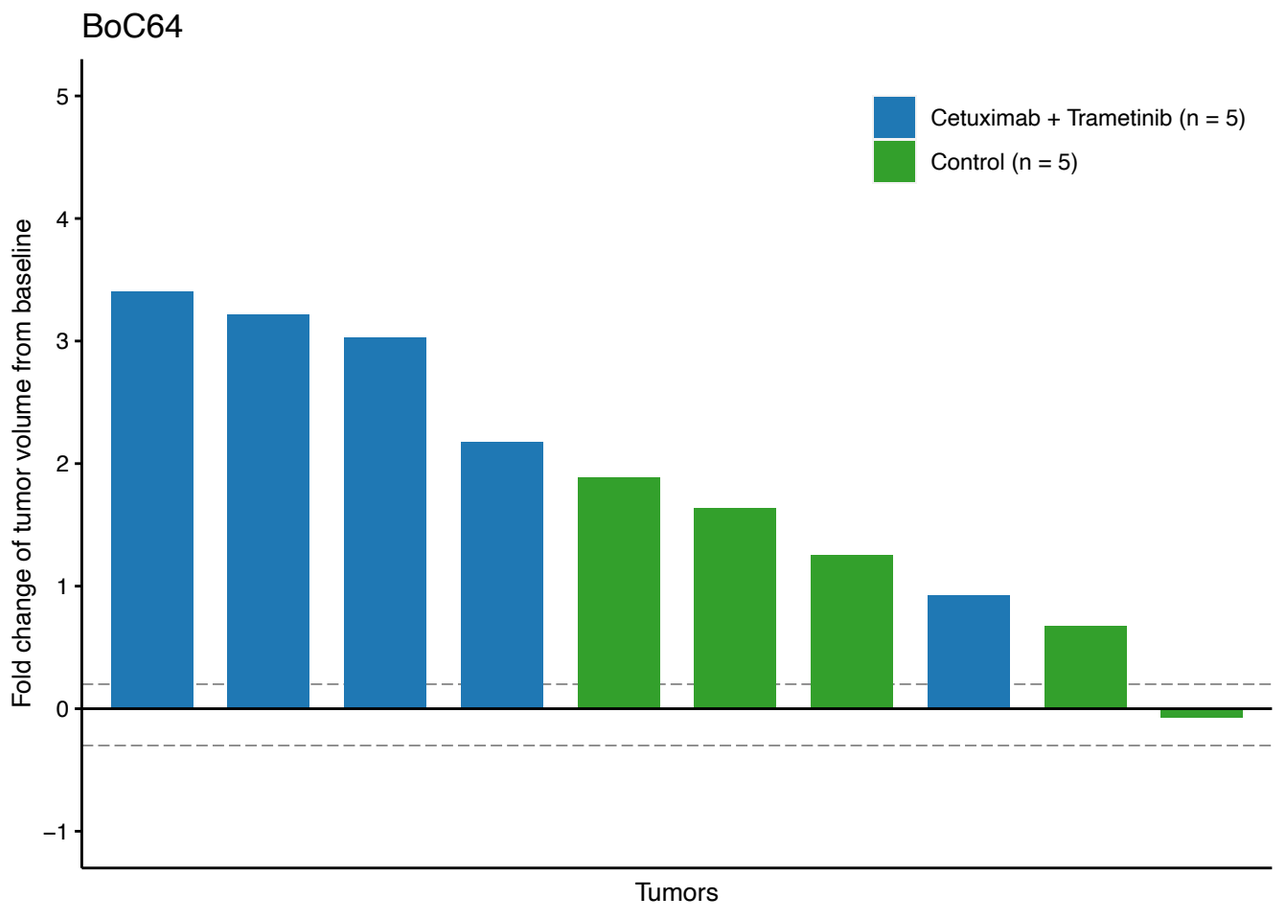

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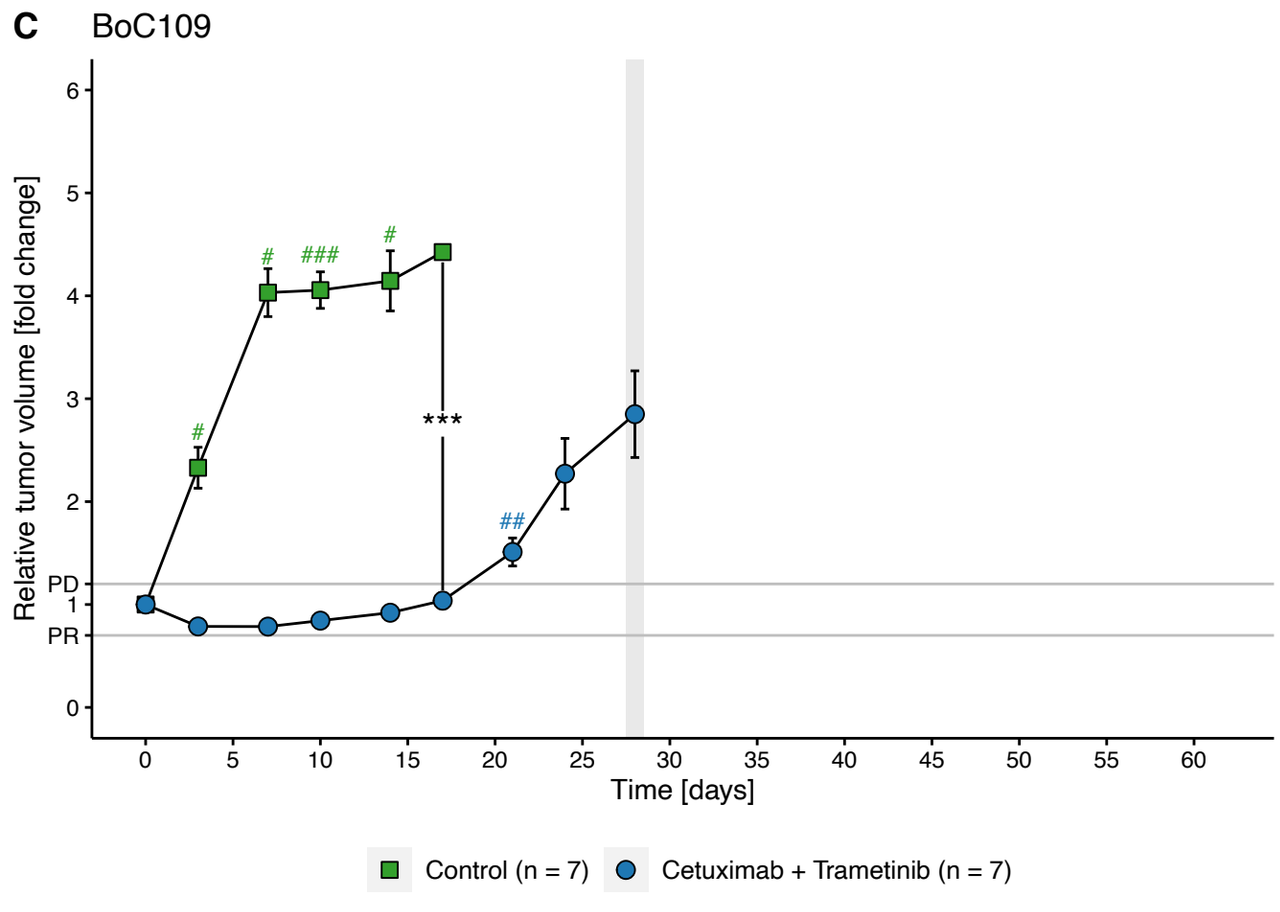

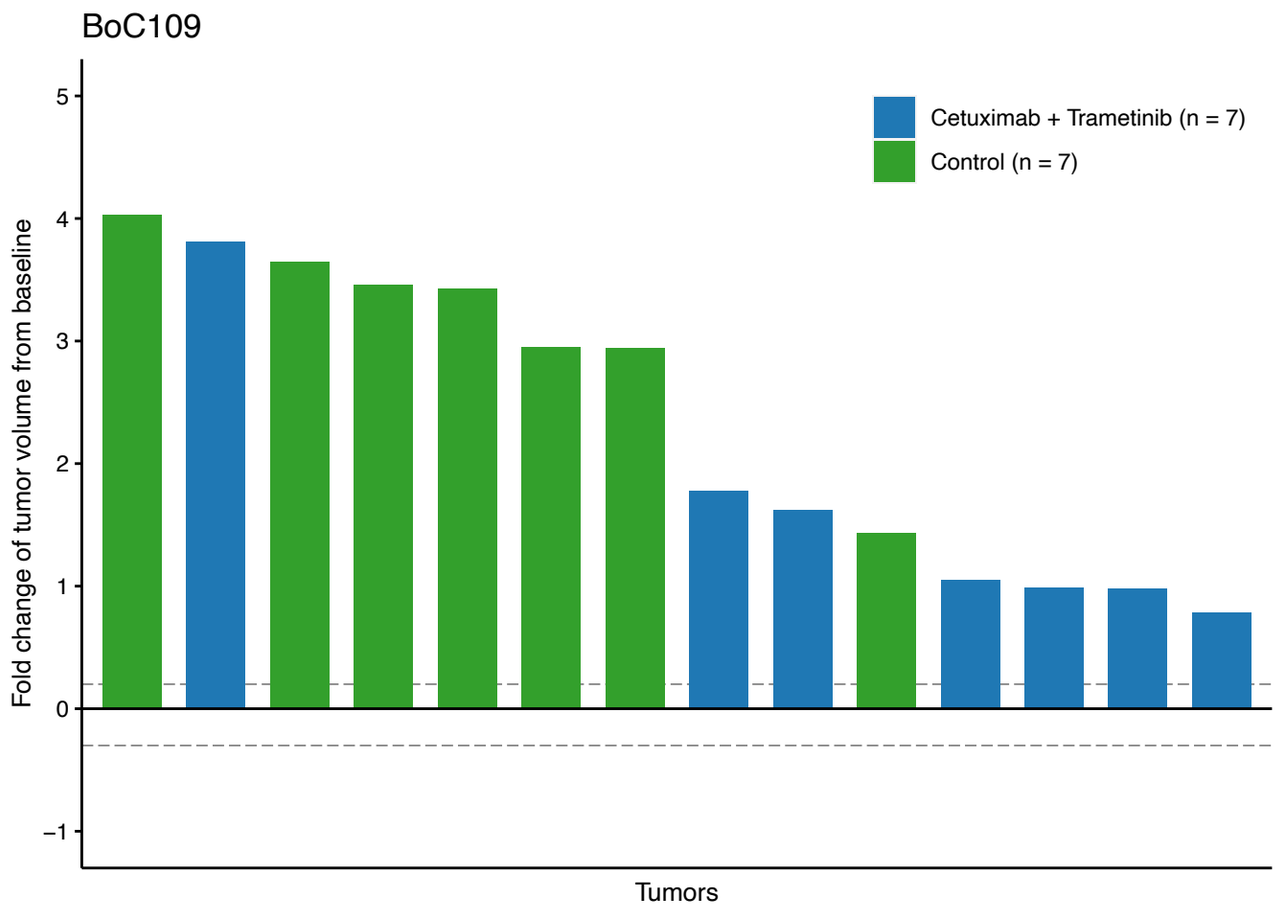

Supplement Figure 7

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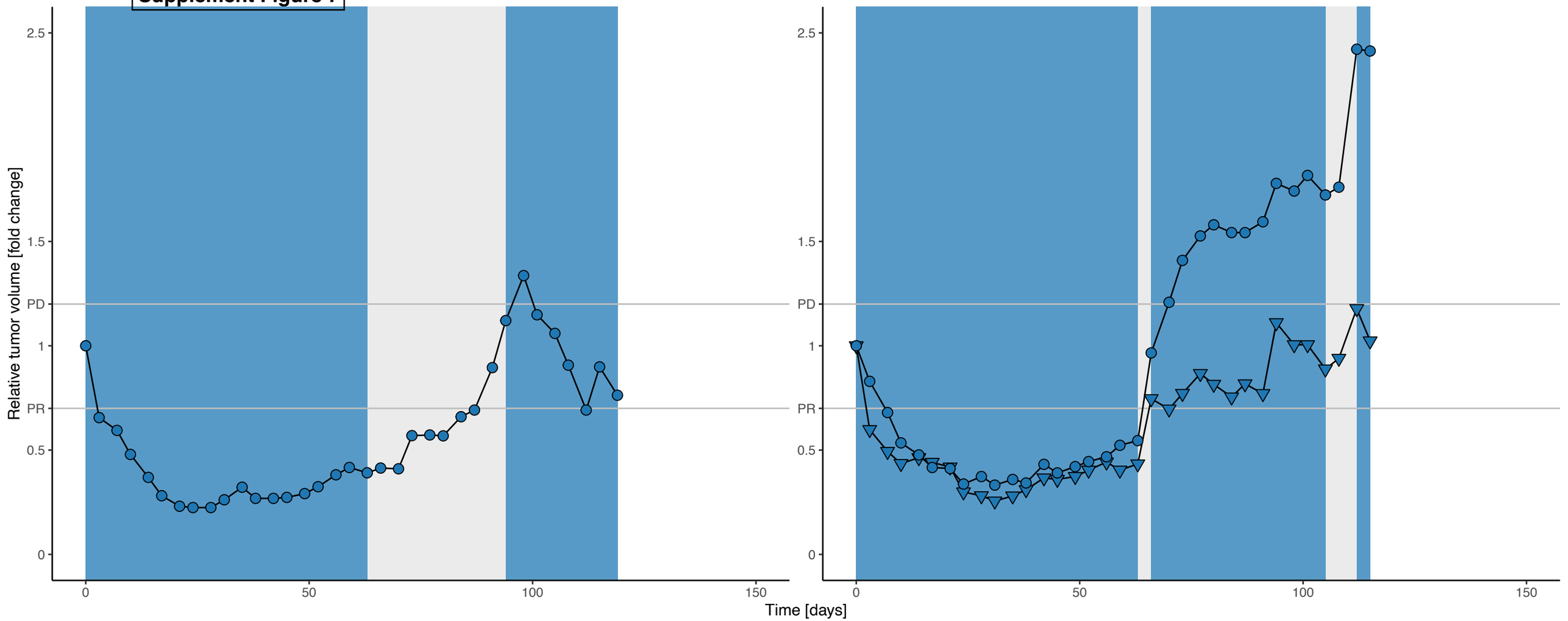

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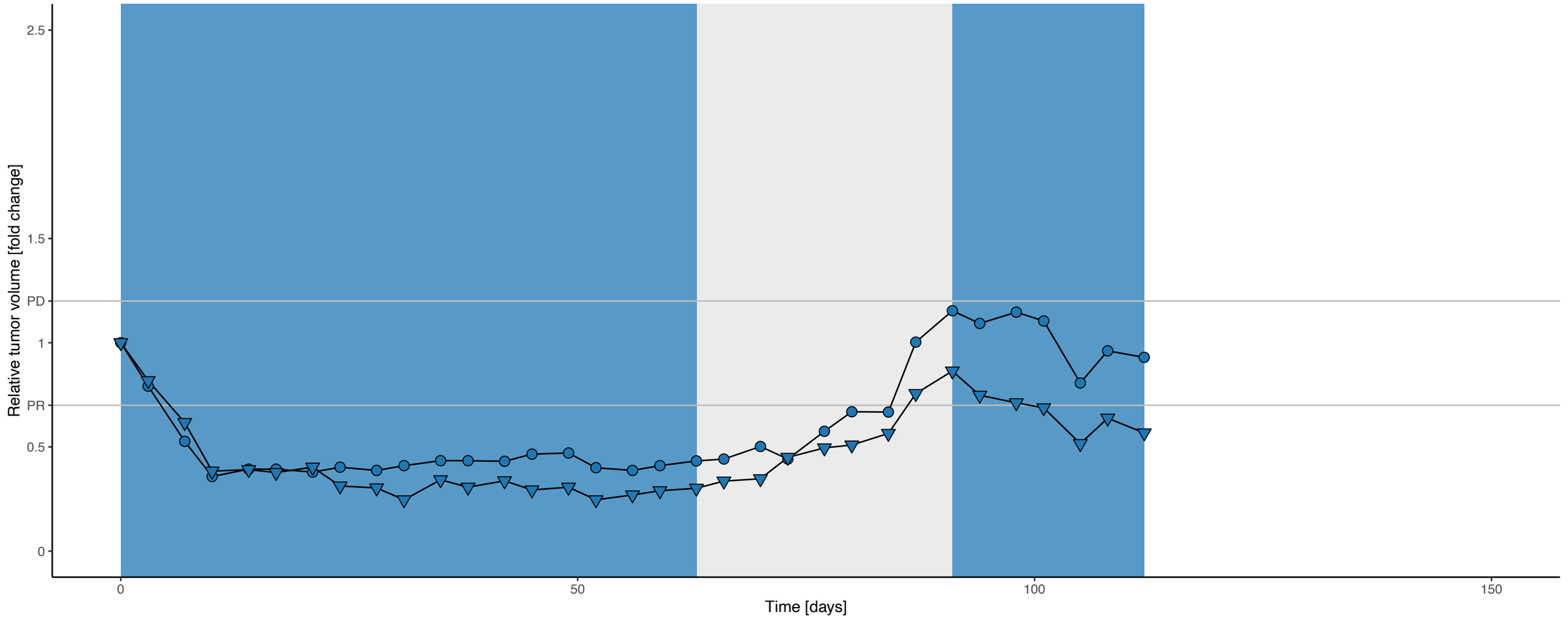
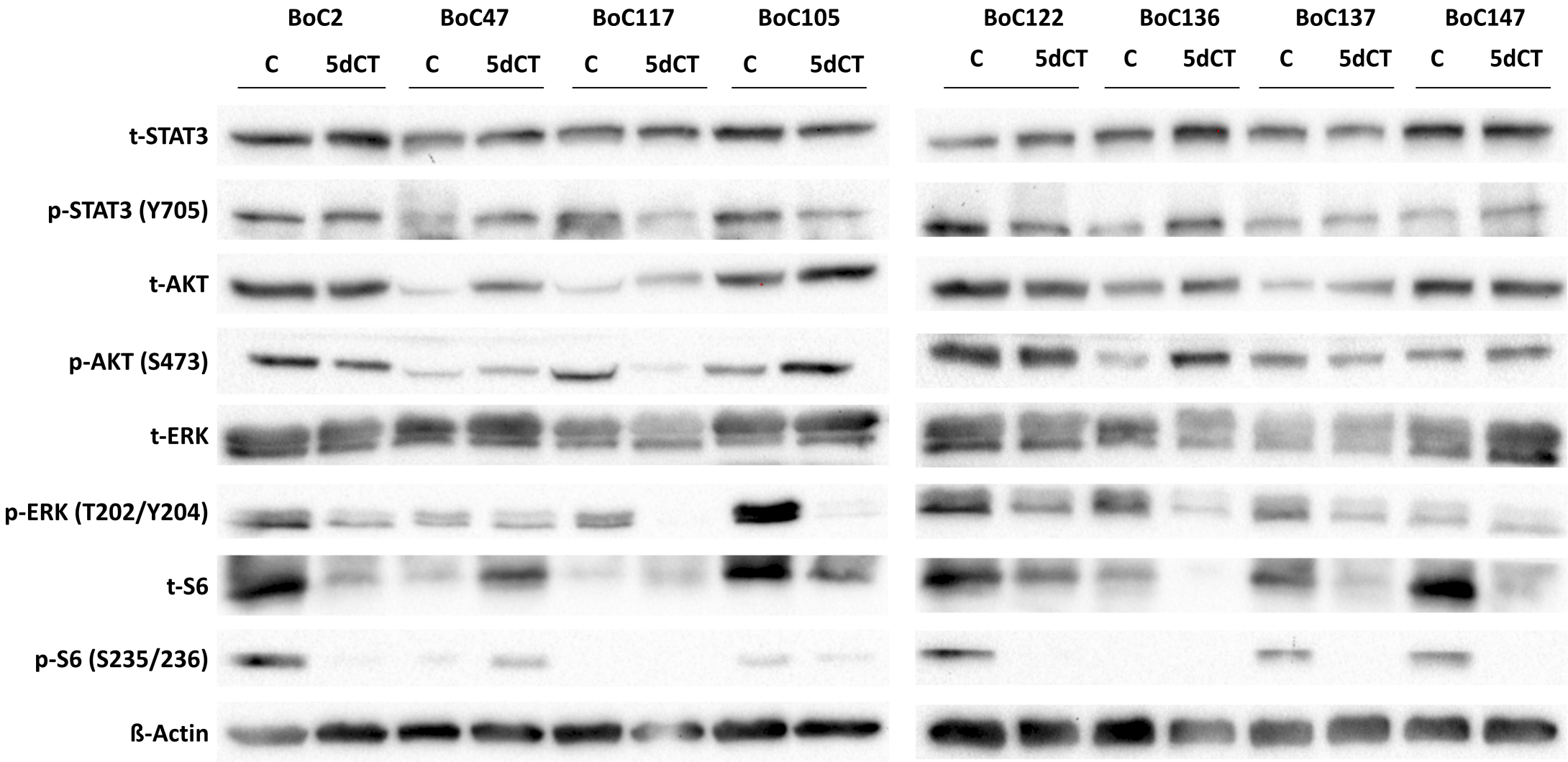
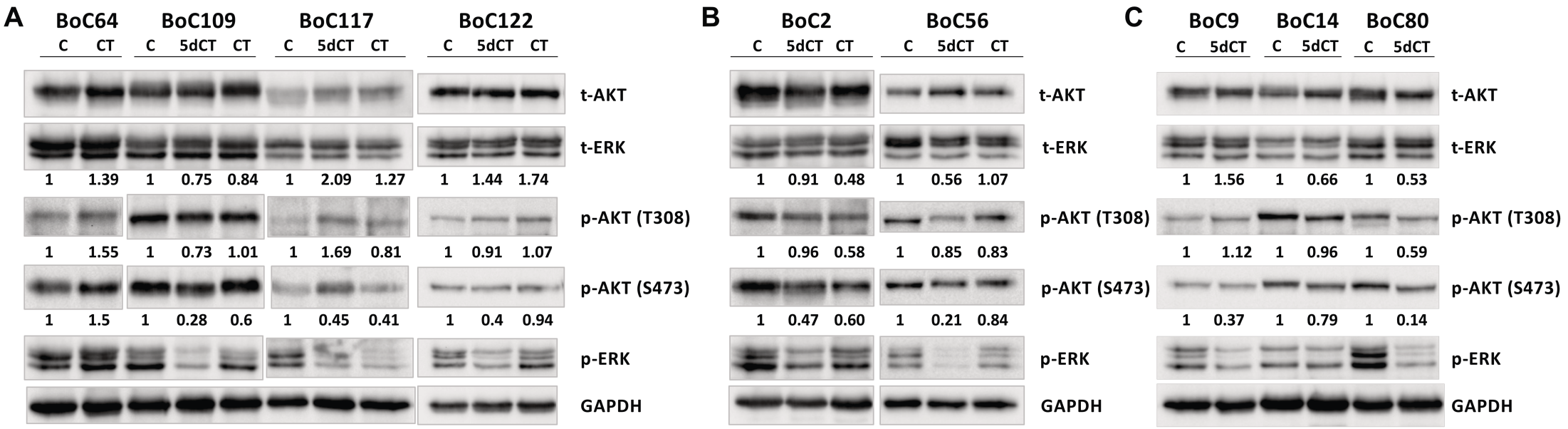
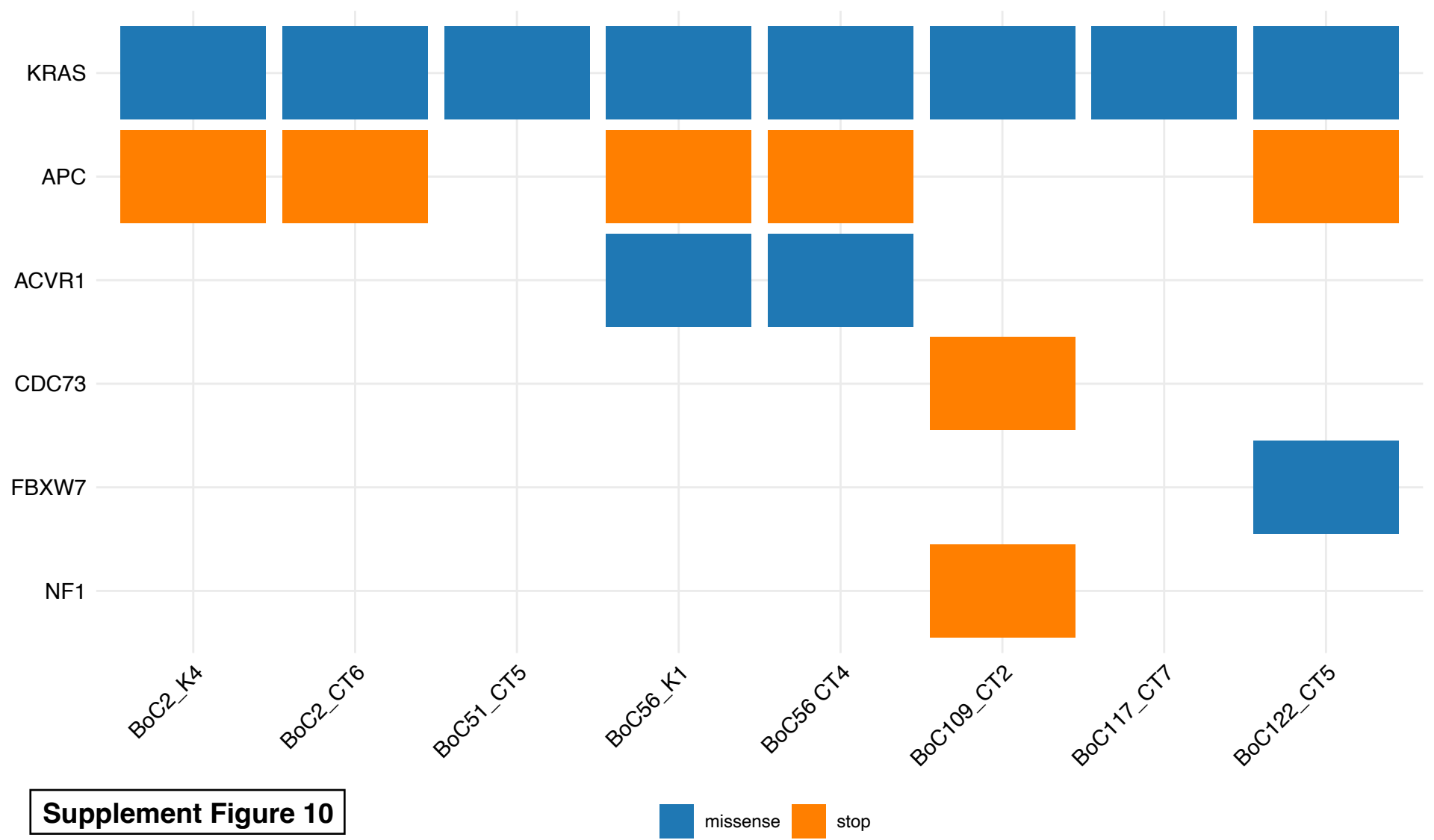


Supplement Figure 8



Supplement Figure 9





Supplement Figure 10