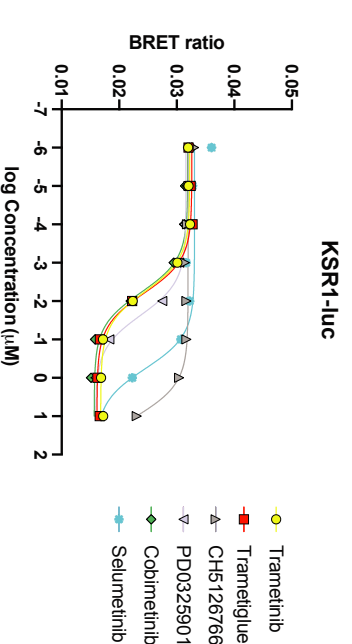
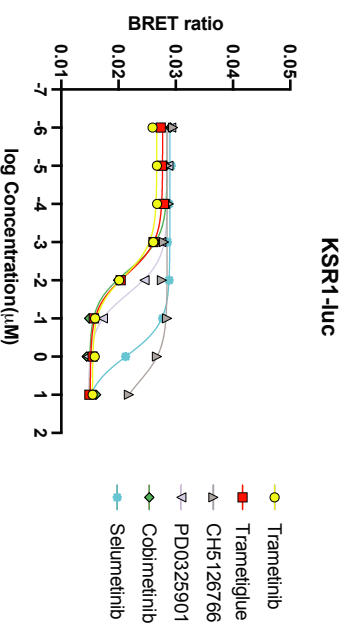
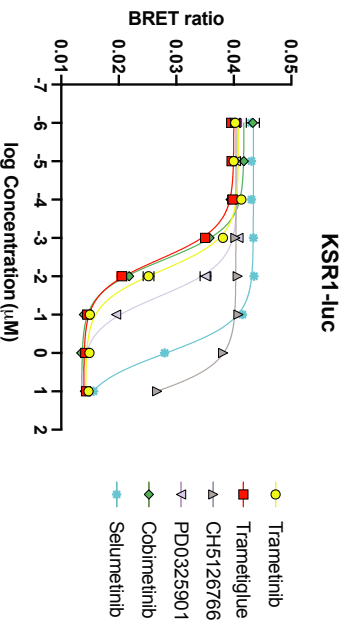
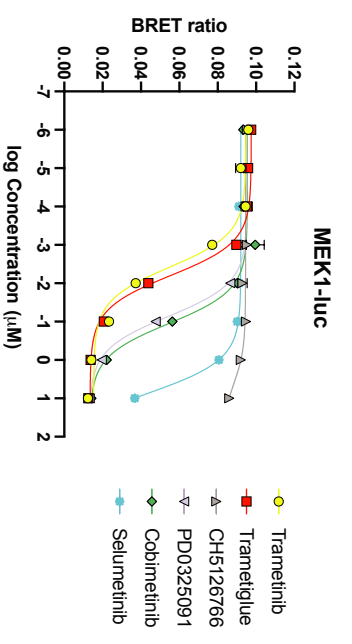
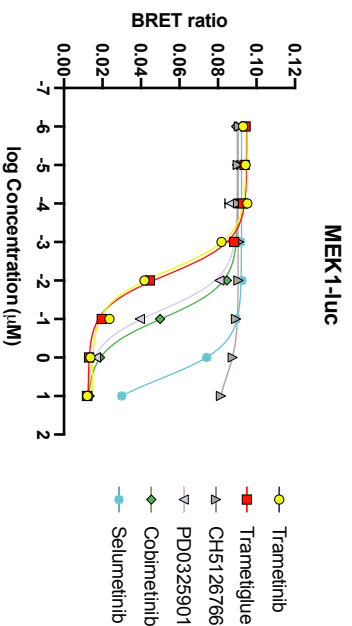
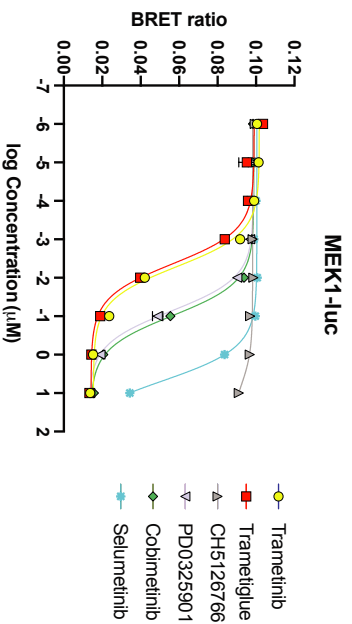


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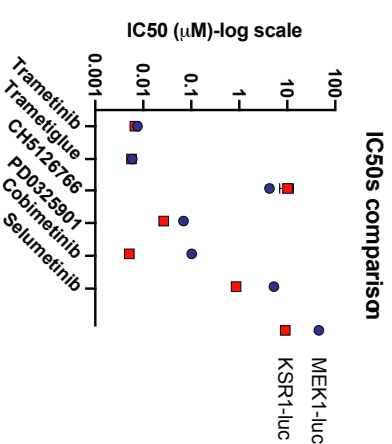
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Trametinib (μM)	Independent replicate 1				Independent replicate 2				Independent replicate 3				
	plate 1	plate 2	plate 3	plate 4	plate 1	plate 2	plate 3	plate 4	plate 1	plate 2	plate 3	plate 4	plate 5
KSR1-luc	0.007069	0.007545	0.005778	0.00464	0.00771	0.00725	0.006874	0.01	0.005647	0.005176	0.005979	0.004772	0.008716
MEK1-luc	0.005249	0.006208	0.006681	0.01084	0.005434	0.005319	0.01058	0.01844	0.003834	0.004926	0.00738	0.00731	0.006859

KSR1(μM)	Ind. Rep			Average	S.E.M
	Ind. Rep 1	Ind. Rep 2	Ind. Rep 3		
Trametinigluce	0.0441	0.0463	0.0312	0.0405	0.0047
CH5126766	15.5500	4.5570	10.4500	10.1857	3.1762
PD0325901	0.0337	0.0226	0.0236	0.0266	0.0035
Cobimetinib	0.0038	0.0056	0.0060	0.0051	0.0007
Selumetinib	1.0340	0.9685	0.5851	0.8625	0.1400

MEK1(μM)	Ind. Rep			Average	S.E.M
	Ind. Rep 1	Ind. Rep 2	Ind. Rep 3		
Trametinigluce	0.0352	0.0296	0.0300	0.0316	0.0018
CH5126766	6.1620	2.8820	3.8050	4.2830	0.9766
PD0325901	0.0748	0.0588	0.0746	0.0694	0.0053
Cobimetinib	0.0987	0.0961	0.1137	0.1028	0.0055
Selumetinib	4.9150	3.6600	7.3450	5.3067	1.0816



Plotted data and error bars represent the mean and SEM of three technical replicates except for Trametinib, which is four technical replicates

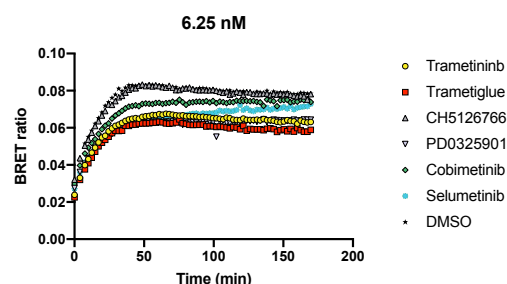
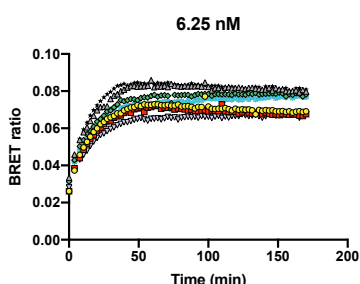
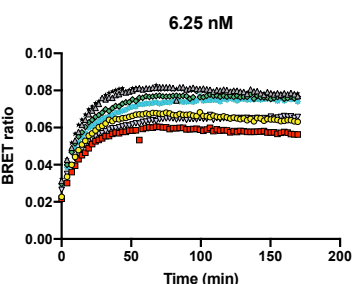
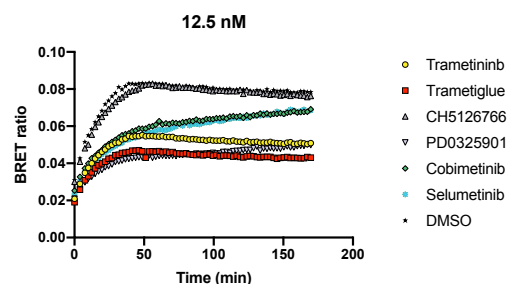
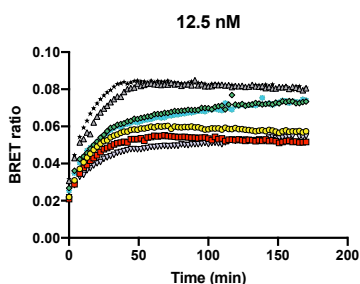
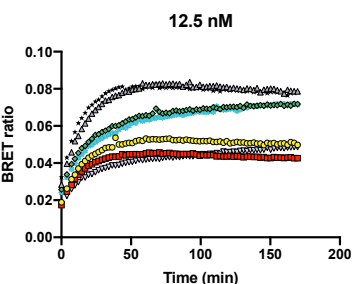
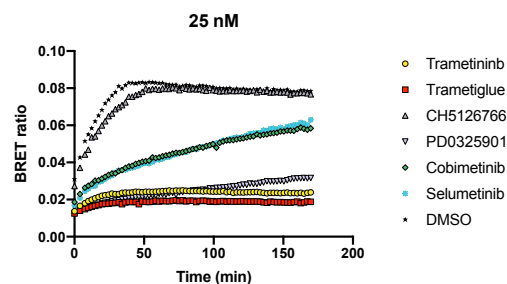
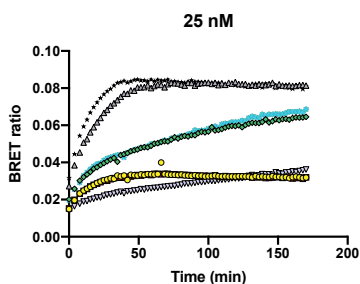
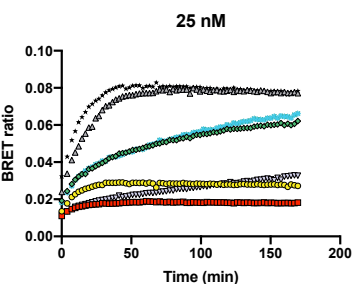
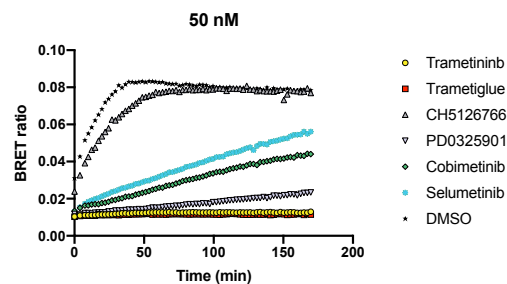
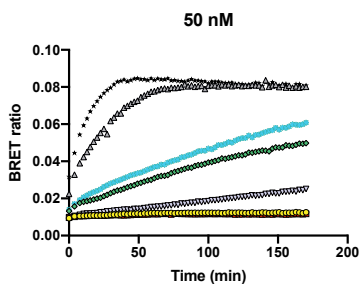
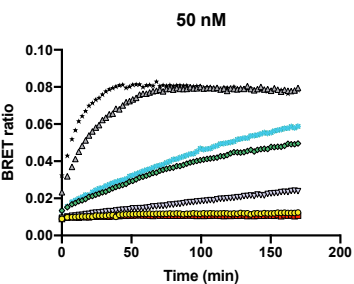
Washout on MEK1-luc reporter

Cells were pre-treated with the indicated concentration for each MEKi, washed with Opti-MEM, and BRET signal was measured for 175 min upon the addition of Tram-bo and the Nanoluc substrate furimazine. Related to Figure 2. Each independent replicate was conducted in technical duplicates.

Independent Replicate 1

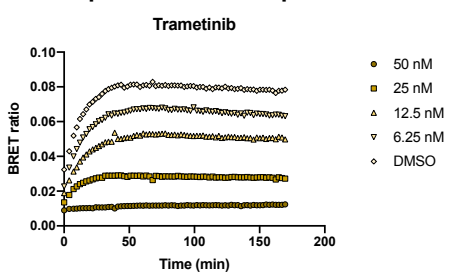
Independent Replicate 2

Independent Replicate 3

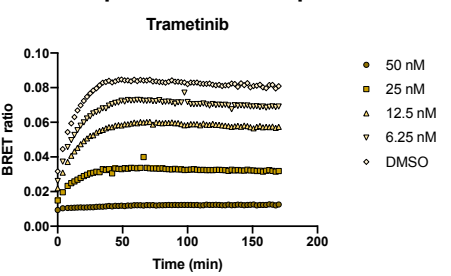


Washout on MEK1-luc reporter: data replotted from page 3 as a function of concentration

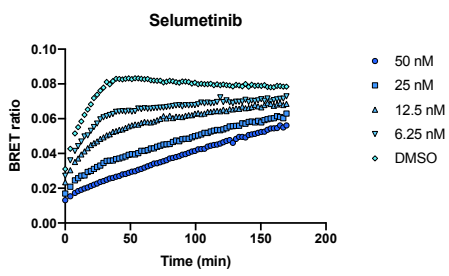
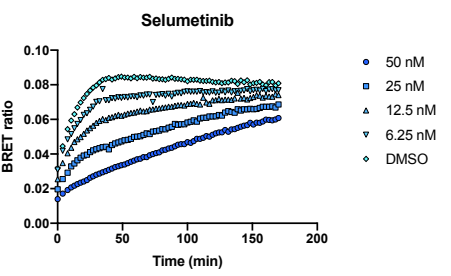
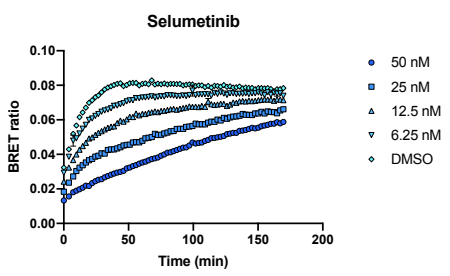
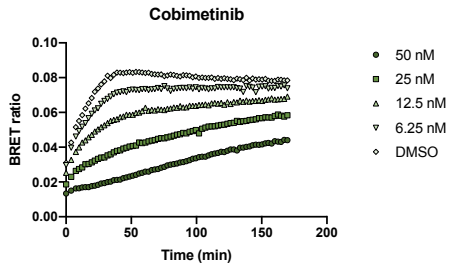
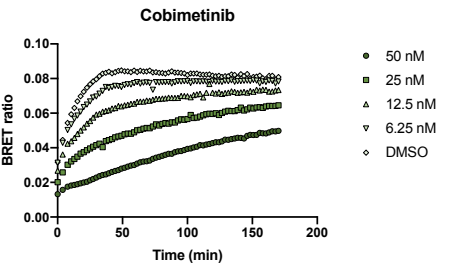
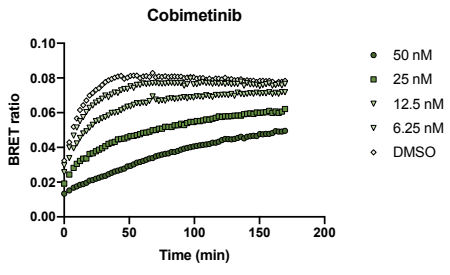
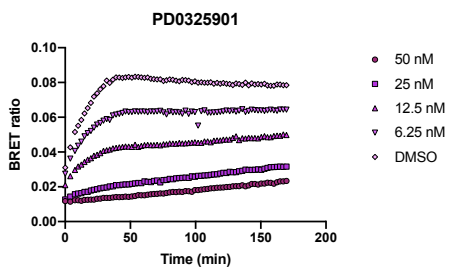
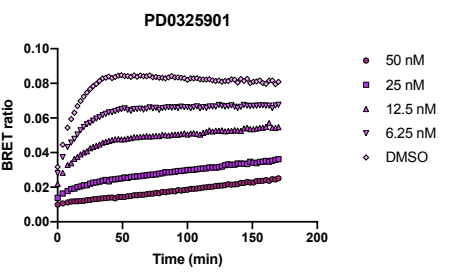
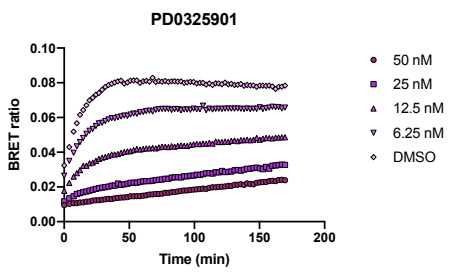
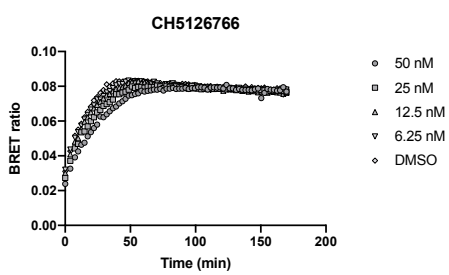
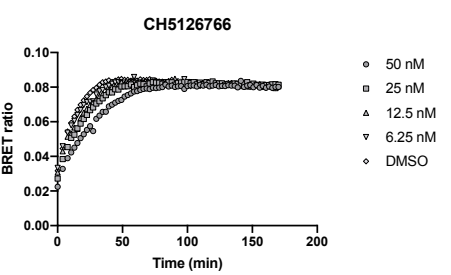
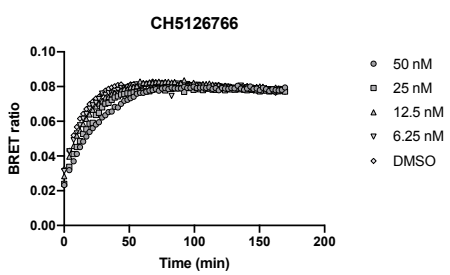
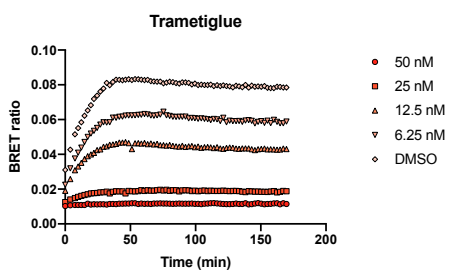
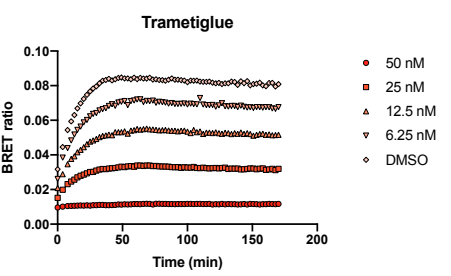
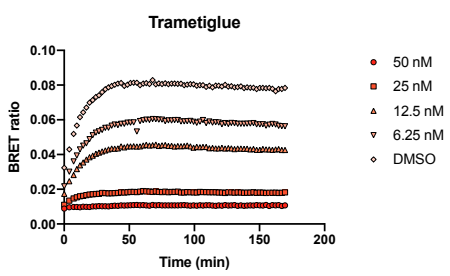
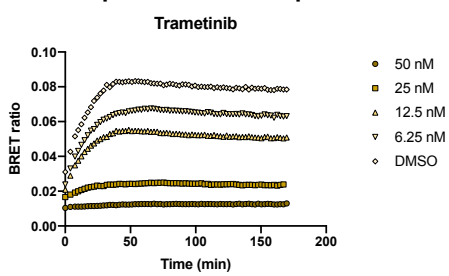
Independent Replicate 1



Independent Replicate 2



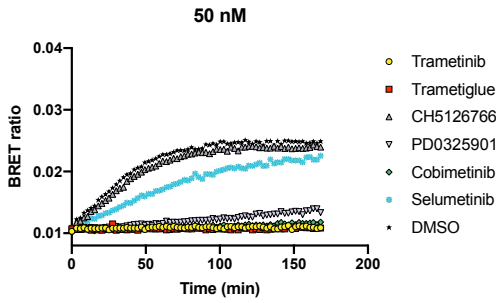
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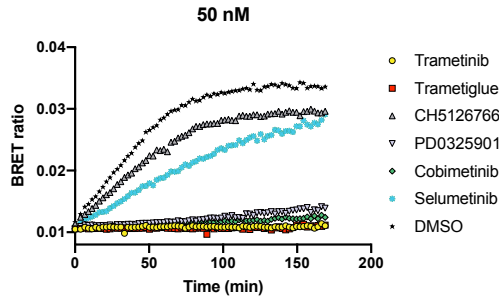
Washout on KSR1-luc reporter

Cells were pre-treated with the indicated concentration for each MEKi, washed with Opti-MEM, and BRET signal was measured for 175 min upon the addition of Tram-bo and the Nanoluc substrate furimazine. Related to Figure 2. Each replicate was conducted in technical duplicates.

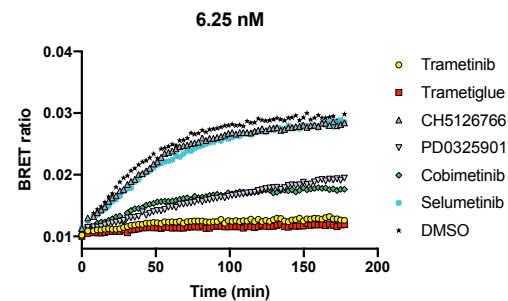
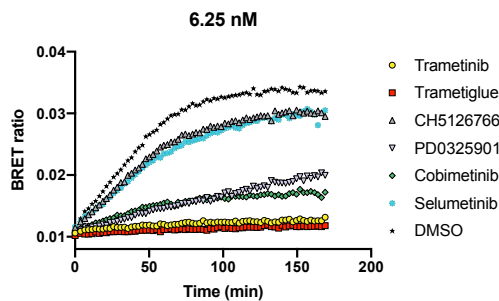
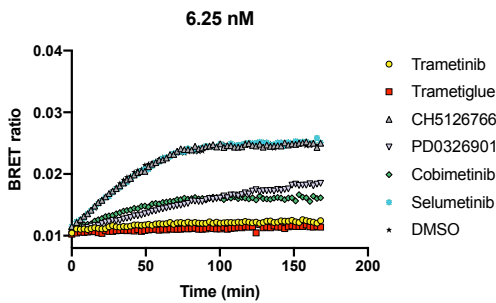
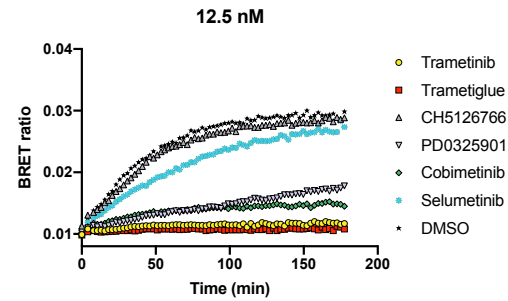
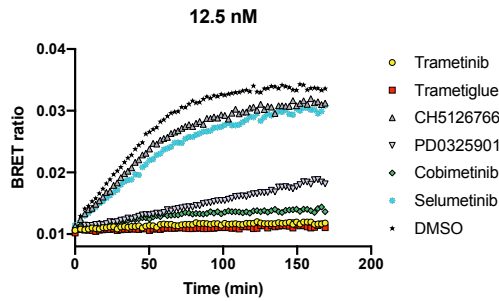
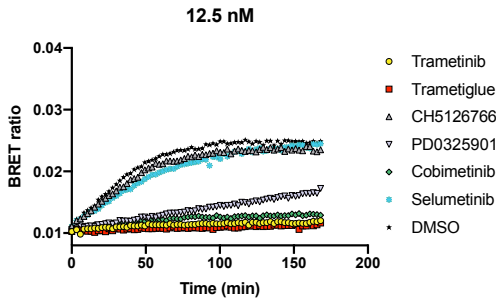
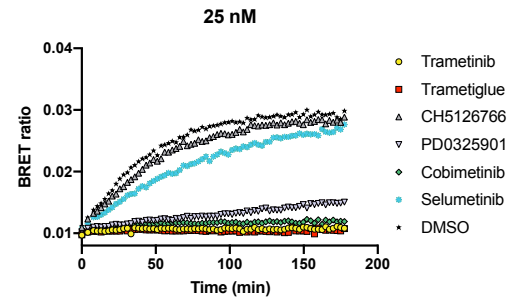
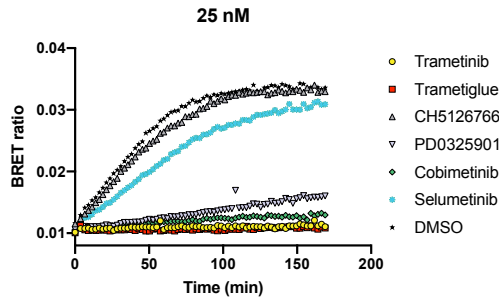
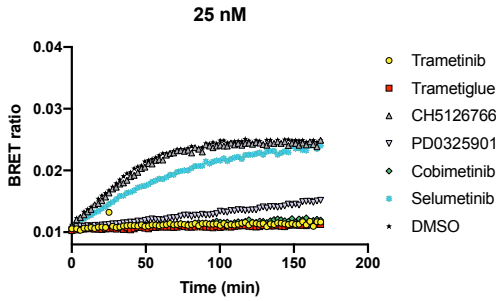
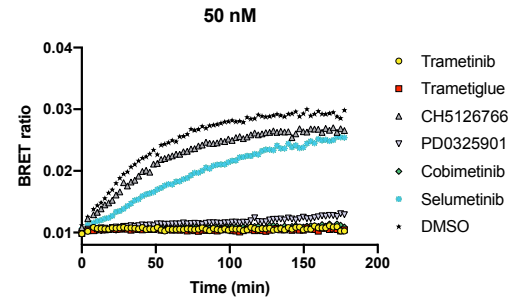
Independent Replicate 1



Independent Replicate 2



Independent Replicate 3



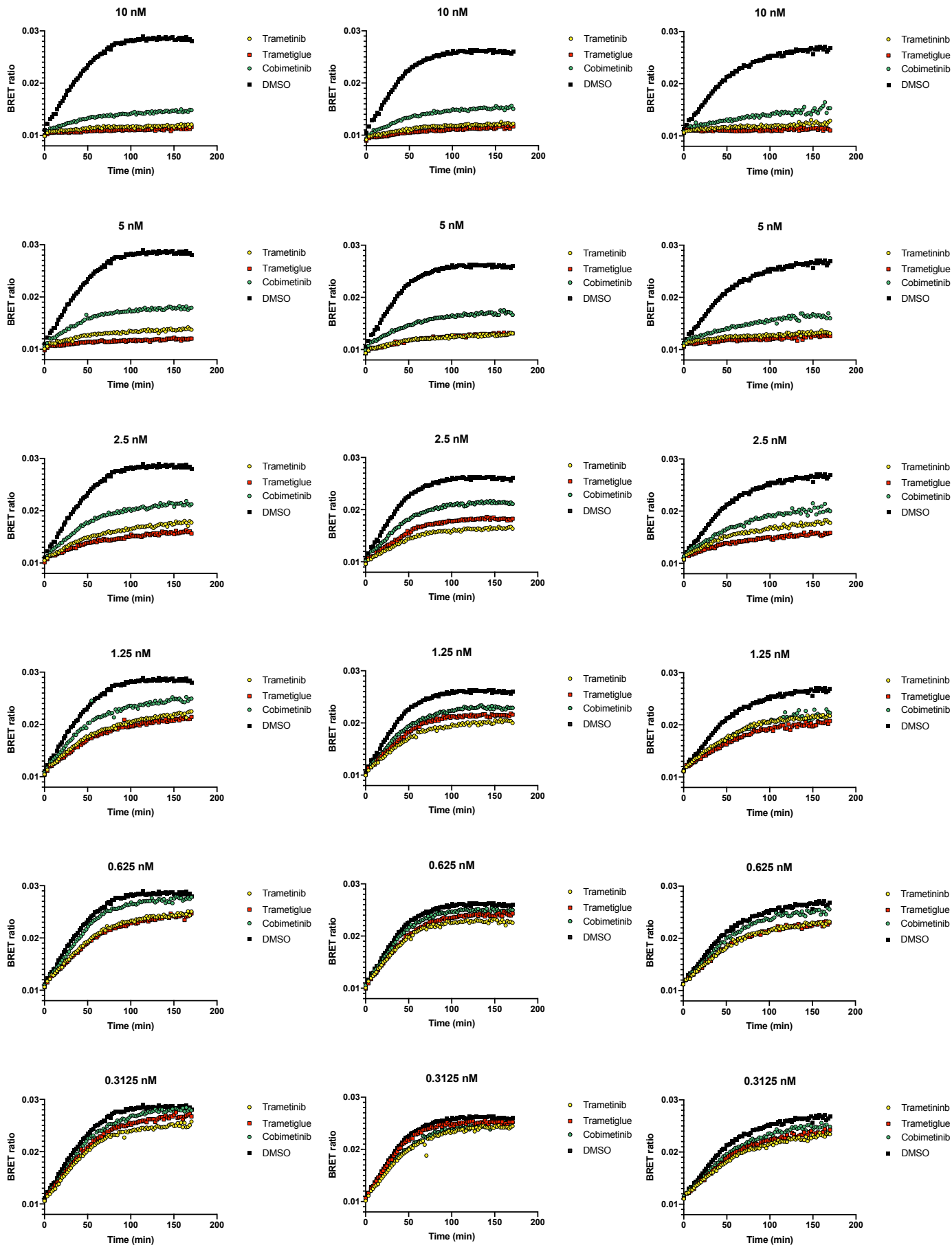
Washout on KSR1-luc reporter

Cells were pre-treated with the indicated concentration for each MEKi, washed with Opti-MEM, and BRET signal was measured for 175 min upon the addition of Tram-bo and the Nanoluc substrate furimazine. Related to Figure 2. Each replicate was conducted in technical duplicates.

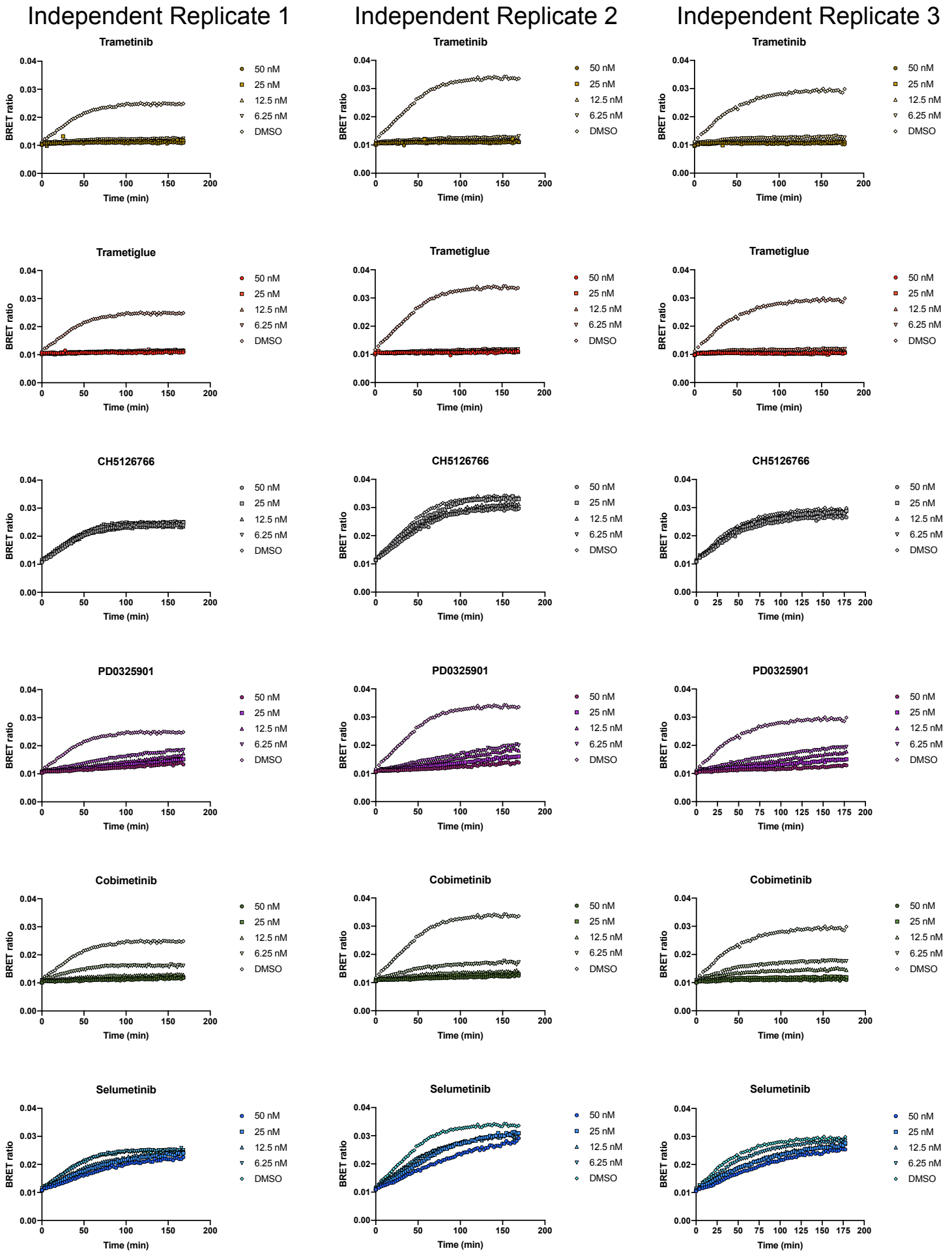
Independent Replicate 1

Independent Replicate 2

Independent Replicate 3



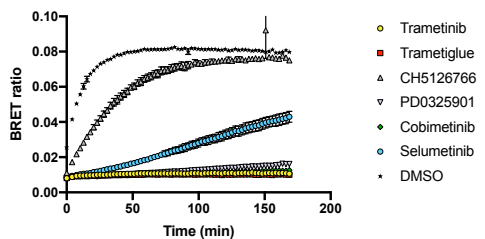
Washout on KSR1-luc reporter: data replotted from page 5 as a function of concentration



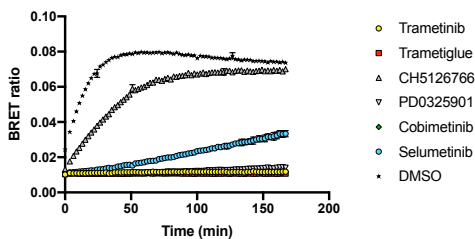
MEK1-luc

wash

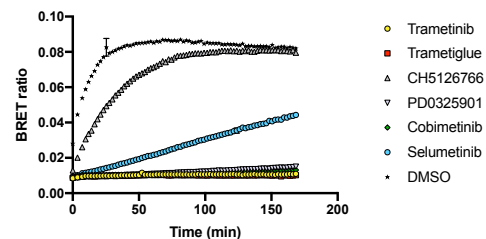
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Independent Replicate 2

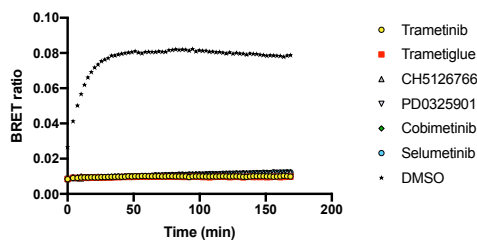


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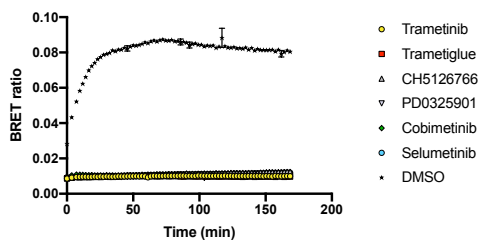


no-wash

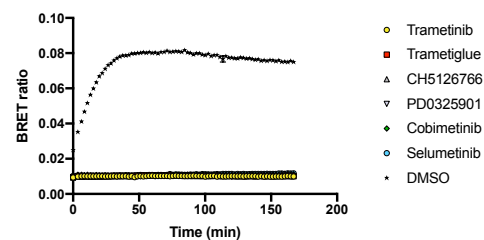
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Independent Replicate 2



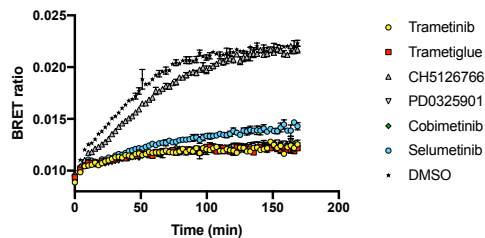
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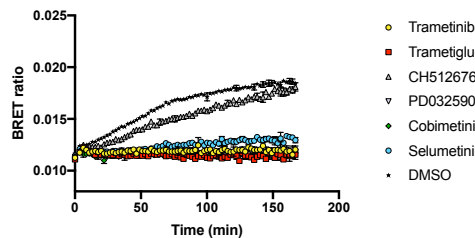
KSR1-luc

wash

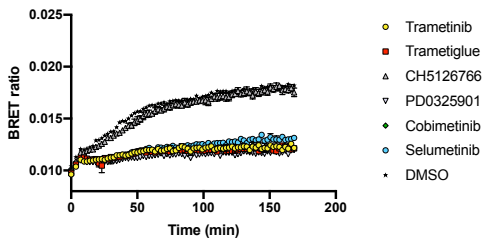
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Independent Replicate 2

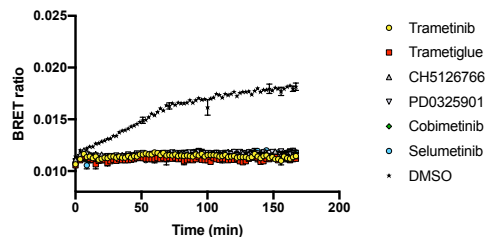


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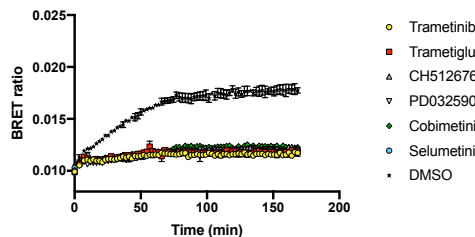


no-wash

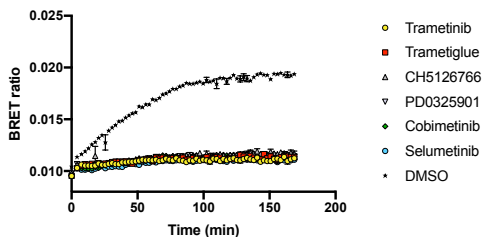
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Independent Replicate 2



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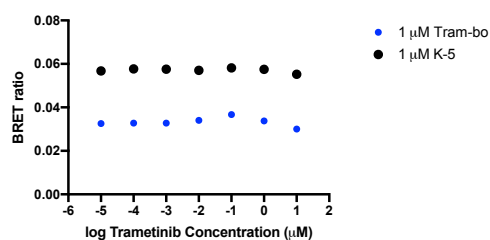


Comparison of tram-bo wash-in buildup curves on cells pre-treated at 15x the IC50 for each MEKi. Cells were either washed with Opti-MEM to remove excess drug (wash) or directly assayed (no-wash) upon the addition of tram-bo and the Nano-luc substrate furimazine. Data and error bars represent the mean and SEM of technical triplicates. The lack of build-up signal under no-wash conditions demonstrates that MEK1-luc and KSR1-luc were completely saturated with each MEKi. However, given the potency of several MEKi (i.e. no build-up over 175 min), lower concentrations of those MEKi were used to distinguish variances in MEKi off-rates or residence time (pages 2 and 4).

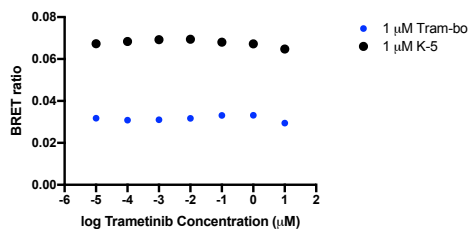
Drug	Saturating Concentration
Trametinib	100 nM
Trametigluce	100 nM
CH5126766	100 μ M
PD0325901	1 μ M
Cobimetinib	2 μ M
Selumetinib	10 μ M

RET-luc + Tram-bo (1 μ M)

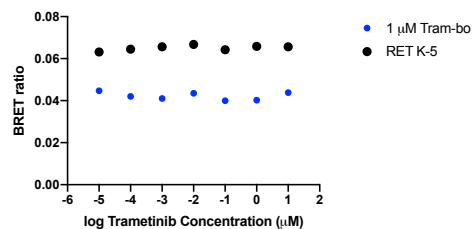
Independent Replicate 1



Independent Replicate 2

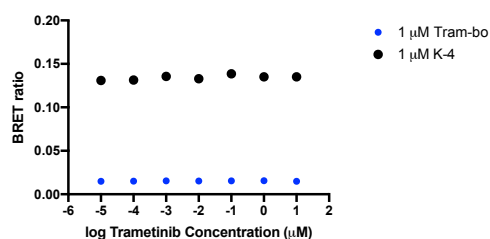


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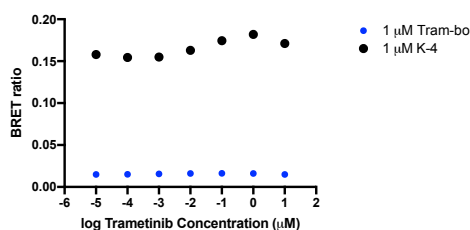


Src-luc + Tram-bo (1 μ M)

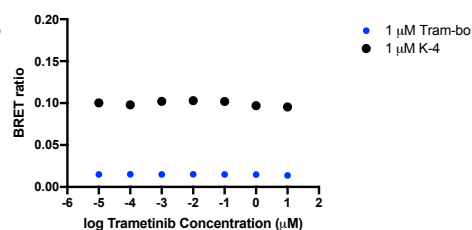
Independent Replicate 1



Independent Replicate 2



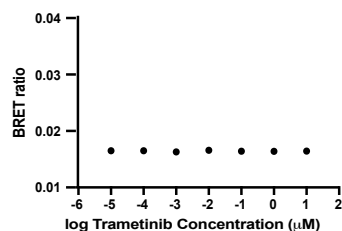
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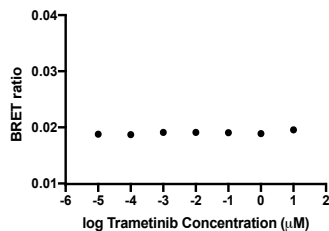
The lack of dose-responses of control kinases (RET and SRC-luc) reveal that tram-bo specifically generates BRET dose on MEK1-luc and KSR1-luc. Related to Extended Data Figure 4. Data represent the average of duplicate technical replicates.

KSR1-luc W781D + Tram-bo (1 μ M)

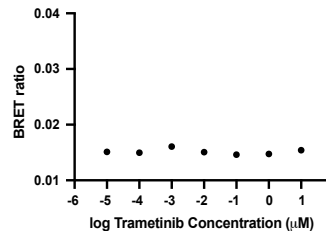
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Independent Replicate 2

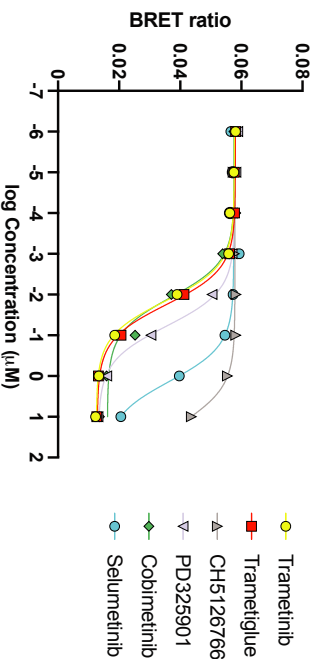


Independent Replicate 3

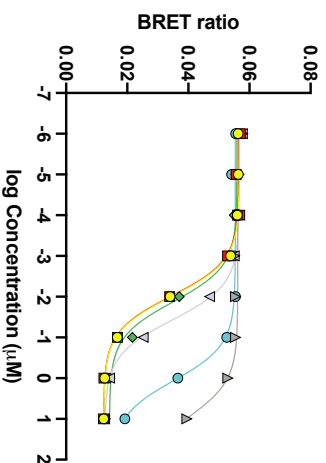


The lack of dose-responses on KSR1-W781D-luc reveals that tram-bo specifically generates BRET dose on on the MEK1-KSR1-complex. Data represent the average of duplicate technical replicates.

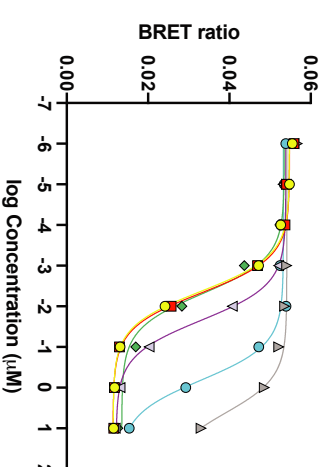
MEK1-luc + KSR1-WT



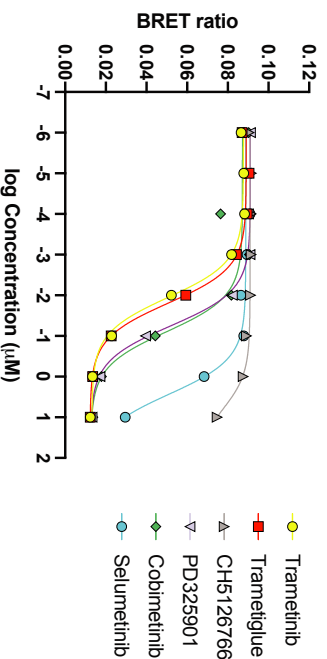
MEK1-luc + KSR1-WT



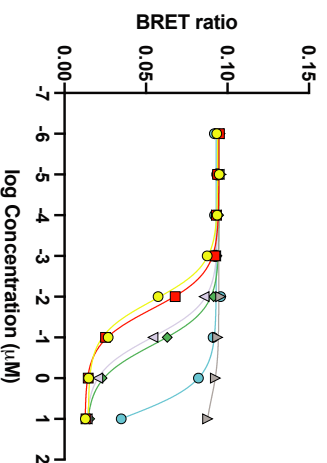
MEK1-luc + KSR1-WT



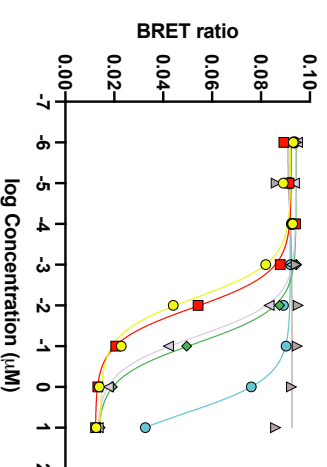
MEK1-luc + KSR1-W781D



MEK1-luc + KSR1-W781D



MEK1-luc + KSR1-W781D



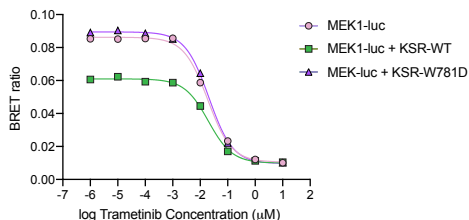
MEK1-luc + KSR1-WT (μM)	ind. rep. 1	ind. rep. 2	ind. rep. 3	Average	SEM
Trametinib	0.00429	0.01003	0.01455	0.00962333	0.00296878
Trametigluce	0.004731	0.009799	0.01782	0.01078333	0.00381039
CH5126766	4.167	8.029	12.25	8.14866667	2.33412813
PD0325901	0.0224	0.03817	0.06024	0.04027	0.01097382
Cobimetinib	0.004868	0.01346	0.01183	0.01005267	0.00263469
Selumetinib	0.6553	1.162	1.357	1.0581	0.20911889

MEK1-luc + KSR1-W781D (μM)	ind. rep. 1	ind. rep. 2	ind. rep. 3	Average	SEM
Trametinib	0.01149	0.01246	0.006539	0.010163	0.00183351
Trametigluce	0.01574	0.02021	0.0114	0.01578333	0.00254332
CH5126766	6.207	3.123	N/A	4.665	1.25903773
PD0325901	0.05739	0.1017	0.05819	0.07242667	0.01463849
Cobimetinib	0.07962	0.1551	0.08446	0.10639333	0.02439338
Selumetinib	2.738	9.274	4.336	5.44933333	1.96718558

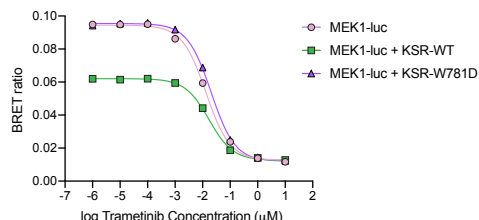
Plotted data represent the mean of duplicate technical replicates

Comparison of Trametinib dose-response curves on different MEK1 co-expressions (Related to Extended Data Figure 5). Data and error bars represent the mean duplicate technical replicates.

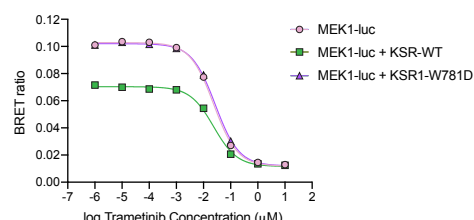
Independent Replicate 1



Independent Replicate 2

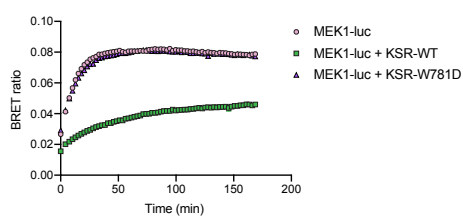


Independent Replicate 3

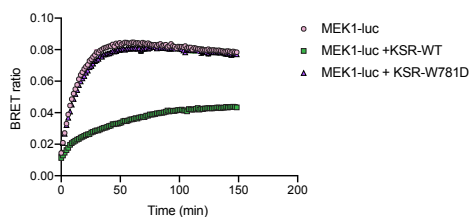


Comparison of Tram-bo wash-in buildup curves on different MEK1 co-expressions (Related to Extended Data Figure 5) Data and error bars represent the mean and SEM of three technical replicates.

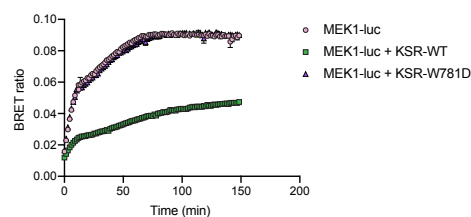
Independent Replicate 1



Independent Replicate 2

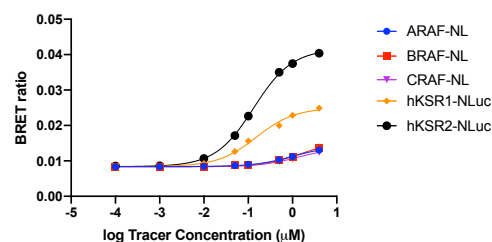


Independent Replicate 3

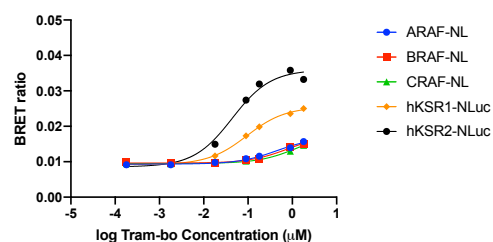


Tram-bo Buildup Curves for ARAF-luc, BRAF-luc, CRAF-luc, hKSR1-luc, and hKSR2-luc (Related to Extended Data Figure 5) Data represent the mean of duplicate technical replicates.

Independent Replicate 1



Independent Replicate 2



Independent Replicate 3

