narrative text

As shown in Fig. 5A, PLX4032 treatment increased *HER3* and *HER2* mRNAs in all 6 *BRAF*- mutant thyroid cancer cell lines tested.

Fig5A

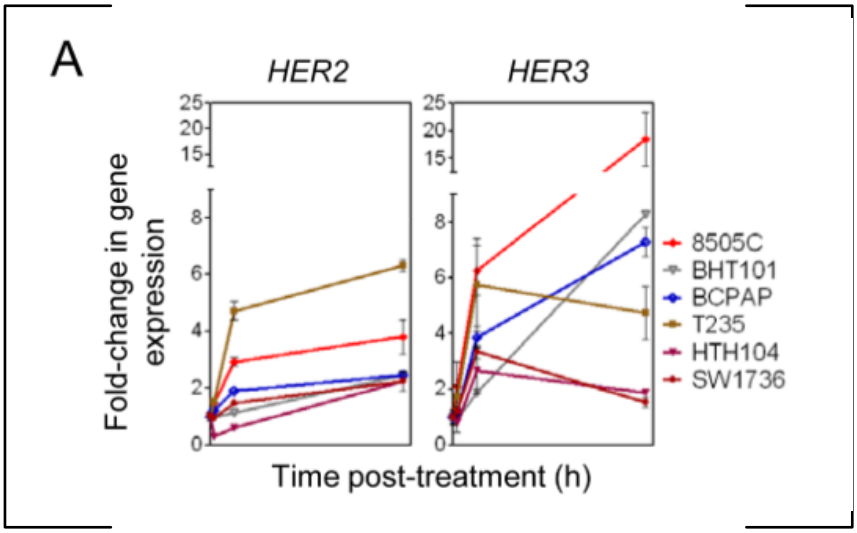
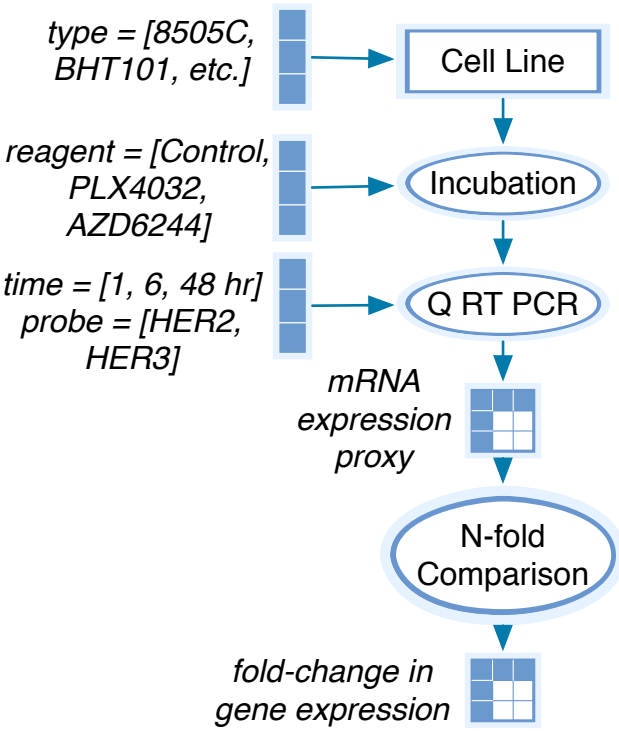


Fig5A Caption

A, a panel of BRAF-mutant thyroid cells was treated with 2 µmol/L PLX4032 for 1, 6, or 48 hours and cell lysates analyzed for expression of HER3 and HER2 by quantitative (qRT-PCR). Points represent fold- change of HER/GAPDH qRT-PCR values of triplicate assays (mean ± SD) over untreated controls.



This model generates this KEfED data structure:

?mRNA expression [?cell-type]
[?reagent]
[?time]
[?probe]

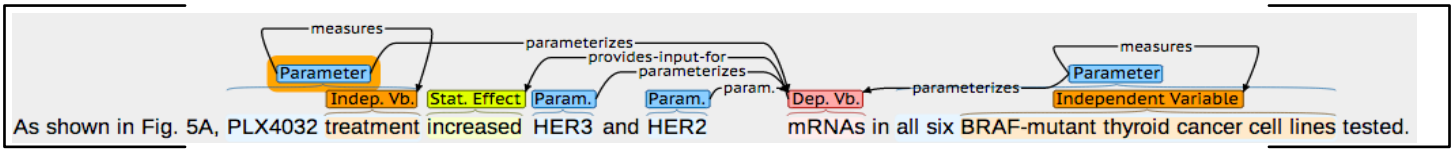
This then provides these data tuples

*mRNA expression [*][PLX4032][*][HER3]*
>>
*mRNA expression [*][Control][*][HER3]*

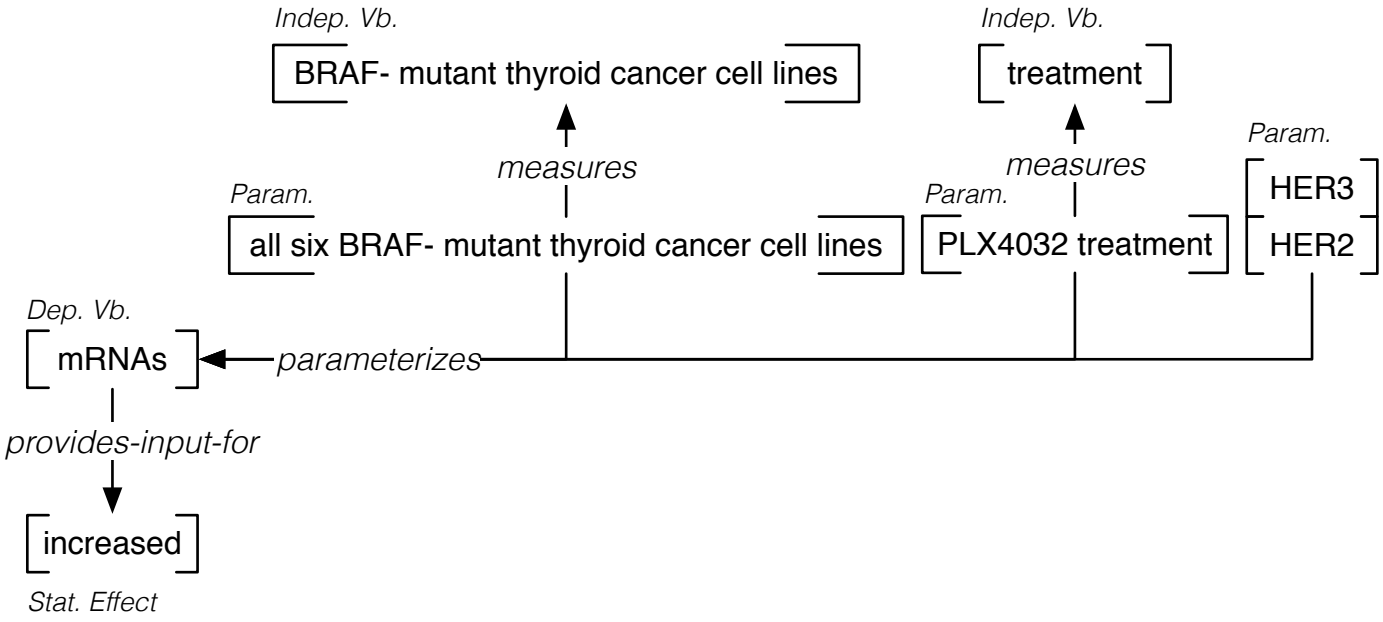
*mRNA expression [*][PLX4032][*][HER2]*
>>
*mRNA expression [*][Control][*][HER2]*

~~~
~~~

annotation



How does this annotation capture the KEfED data structure?



narrative text

The effects of the MEK inhibitor on total HER2, HER3 protein and on pHER3 were dose dependent, and inversely associated with the degree of inhibition of pERK (Fig. 5B)

Fig5B

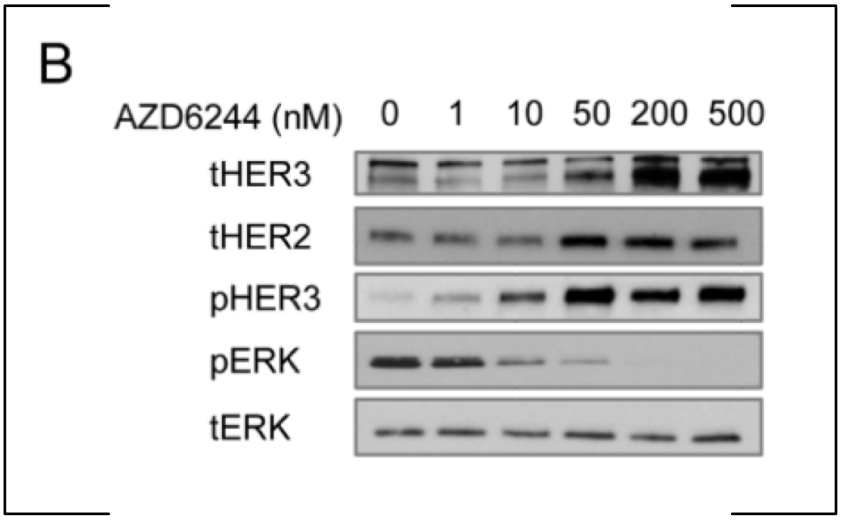
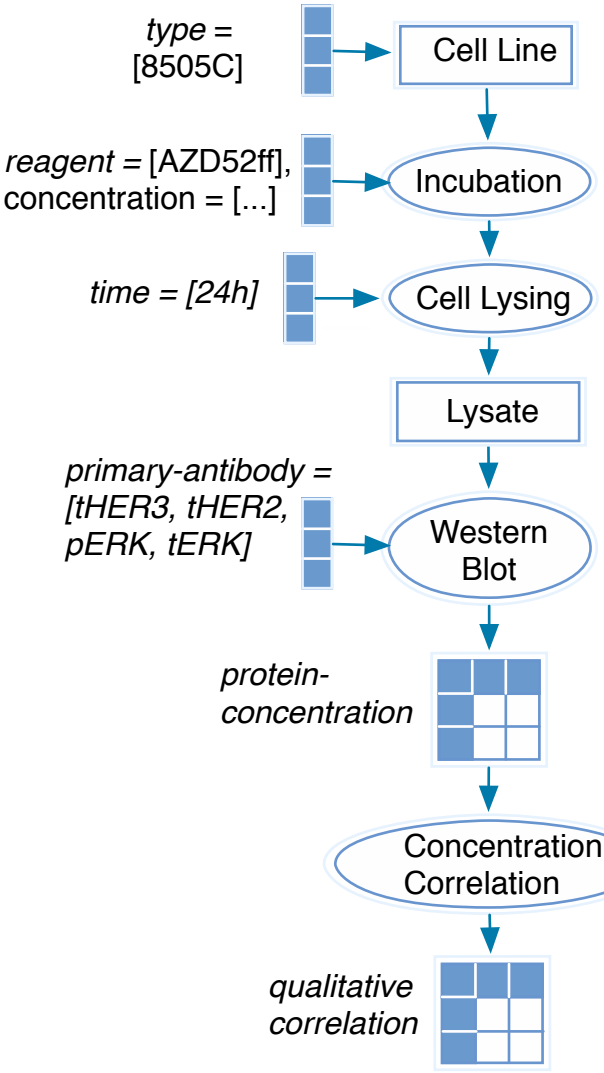


Fig5B Caption

B, 8505C cells were treated with increasing concentrations of AZD6244. Lysates were extracted at 24 h post-treatment and immunoblotted with the indicated antibodies.



This model generates this KEfED data structure:

?protein-concentration [?cell-type]
[?reagent]
[?time]
[?concentration]
[?primary-antibody]

The statement basically says

?protein-concentration [8505C]
[AZD52ff]
[24h]
[0, 1, 10, 50, 200]
[tHER3, tHER2, pHER3]

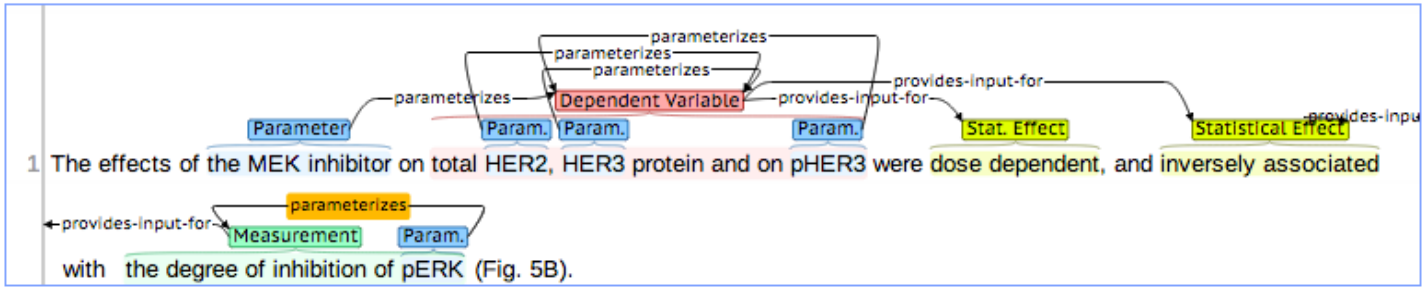
monotonically increases as ?concentration increases

But...

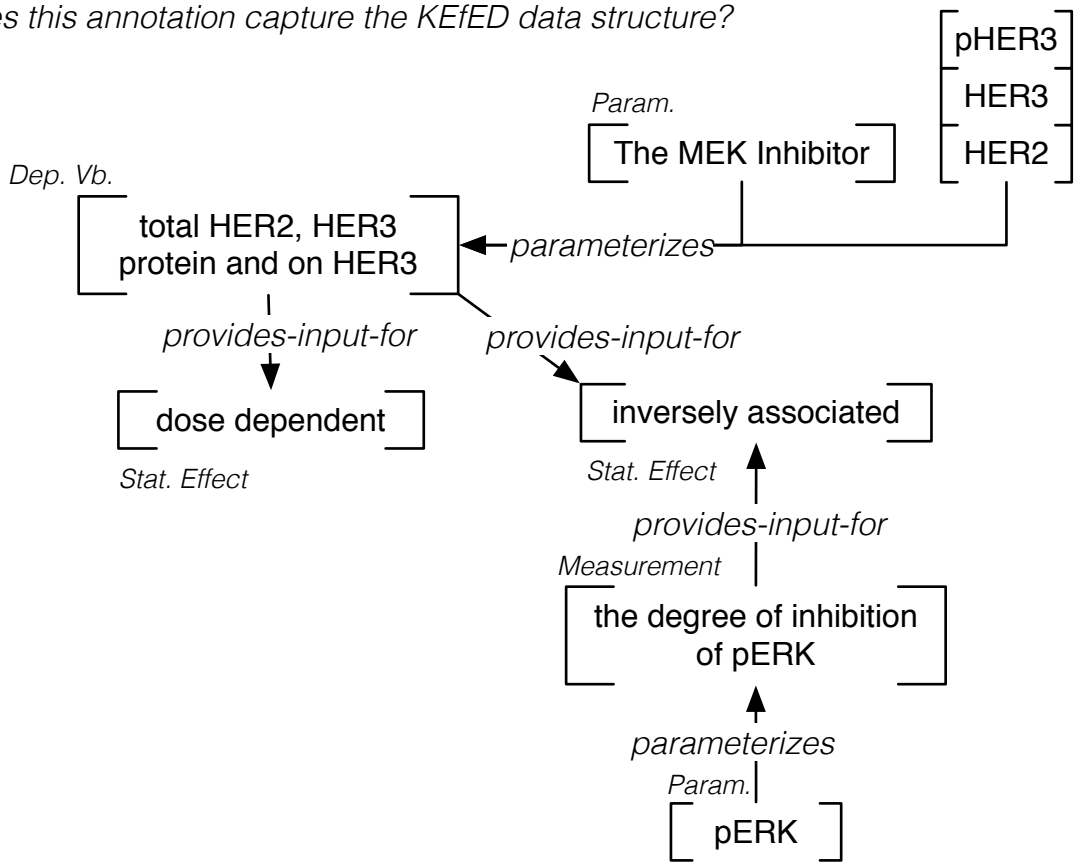
?protein-concentration [8505C]
[AZD52ff]
[24h]
[0, 1, 10, 50, 200]
[pERK]

*monotonically **decreases** as ?concentration increases*

annotation



How does this annotation capture the KEfED data structure?



narrative text

RAF or MEK inhibitors induced luciferase activity of a HER3 promoter construct spanning ~ 1 kb upstream of the transcriptional start site in 8505C cells. Serial deletions identified a minimal HER3 promoter retaining transcriptional response to vemurafenib and AZD6244, which was located between ?401 and ?42 bp (Fig. 5C). This region does not contain any predicted FoxO binding sites. Moreover, PLX4032 led to an increase in phosphorylation of FoxO1/3A between 4?10h after addition of compound (not shown), which is known to promote its dissociation from DNA, and likely discards involvement of these factors as transcriptional regulators of HER3 in response to MAPK pathway inhibition.

Fig5C

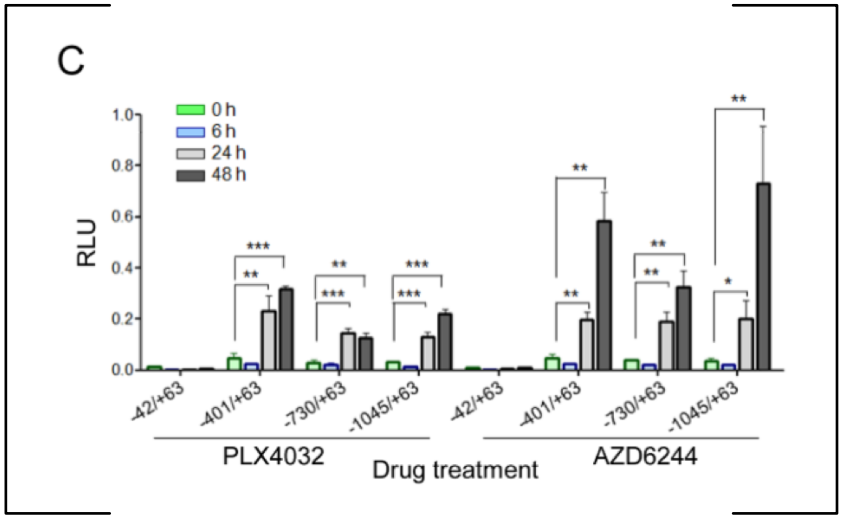
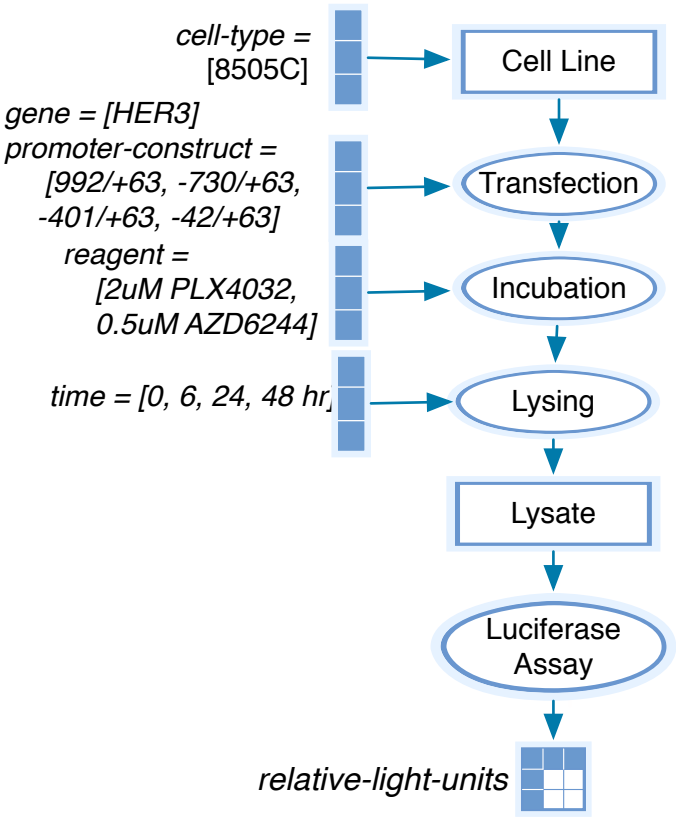


Fig5C Caption

C, luciferase assays of 8505C cells transfected with plasmids containing HER3 promoter- reporter constructs (–992/+63; –730/+63; –401/+63 or –42/+63, relative to transcriptional start site) and pRenilla-CMV, used as transfection normalizing control plasmid. Twelve hours post-transfection, complete media containing 2 μM PLX4032 or 0.5 μM AZD6244 was added to cells. Lysates were obtained at different time points post-treatment (0, 6, 24 and 48 h), and luciferase activity measured. Promoter activity was determined as the ratio between luciferase and renilla, relative to untreated cells. The results shown are the mean +/ – SD of triplicate samples. * p< 0.05, **p < 0.01, ***p < 0.001.



This model generates this KEfED data structure:

?relative-light-units [?cell-type]
[?gene]
[?promoter-construct]
[?reagent]
[?time]

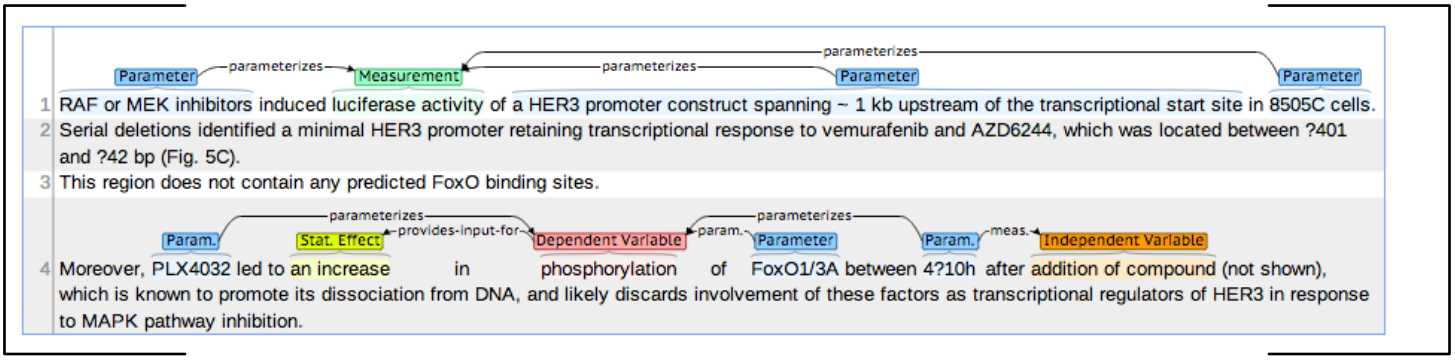
The statement basically says

*?relative-light-units [8505C][HER3][*][PLX4032, AZD6244][*] >> 0*

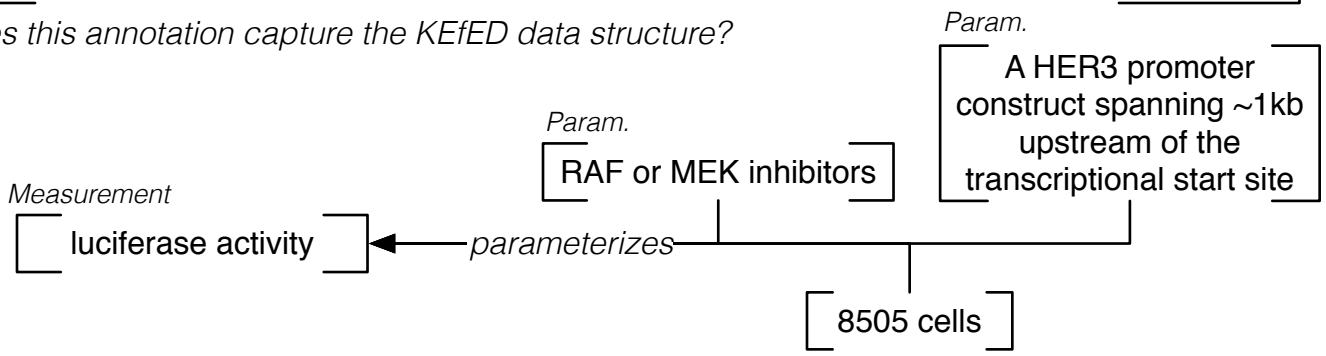
*?relative-light-units [8505C][HER3][-42/+63] [PLX4032, AZD6244][*] == 0* **and**
*?relative-light-units [8505C][HER3][-401/+63] [PLX4032, AZD6244][*] >> 0*

?phosphorylation statement is not about this experiment.... we would need to infer the structure...

annotation



How does this annotation capture the KEfED data structure?



The second statement is interpretive for this type of experiment.

We need to coordinate with BEL / PL / BioPax representation to be able to express this.

narrative text

Silencing of CtBP1, and to a lesser extent CtBP2, increased basal HER3 in 8505C cells, and markedly potentiated the effects of PLX4032 (Fig. 5D and 5E). Knockdown of these factors modestly increased basal and PLX4032-induced HER2 levels, which likely contributes to the remarkable increase in pHER3 we observed (Fig. 5D and 5E).

Fig5D

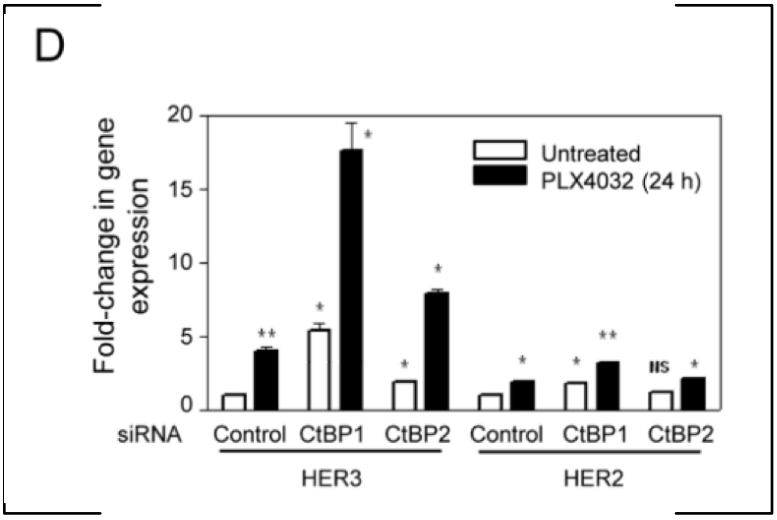
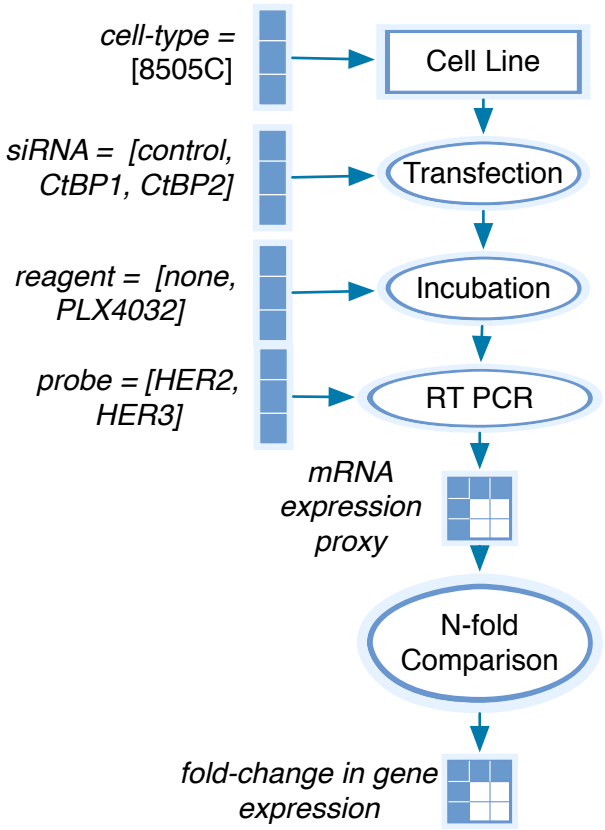


Fig5D Caption

D, 8505C cells were transfected with control, CtBP1 or CtBP2 siRNAs and treated with or without 2 μM PLX4032 for 24 h. Lysates were analyzed for expression of HER3 and HER2 by RT-PCR. Bars represent mean +/– SD of triplicate assays of HER/GAPDH Q-RT-PCR values relative to untreated controls.



This model generates this KEfED data structure:

?fold-change-in-gene-expression [?cell-type]
[?siRNA]
[?reagent]
[?probe]

The statement basically says

*?fold-change-in-gene-expression [8505C][*][*]*
[HER3] >> 1

*?fold-change-in-gene-expression [8505C][*]*
[PLX4032][HER3] >>> 1

*?fold-change-in-gene-expression [8505C][*][*]*
[HER2] > 1

*?fold-change-in-gene-expression [8505C][*]*
[PLX4032][HER3] > 1

narrative text

Silencing of CtBP1, and to a lesser extent CtBP2, increased basal HER3 in 8505C cells, and markedly potentiated the effects of PLX4032 (Fig. 5D and 5E). Knockdown of these factors modestly increased basal and PLX4032-induced HER2 levels, which likely contributes to the remarkable increase in pHER3 we observed (Fig. 5D and 5E).

Fig5E

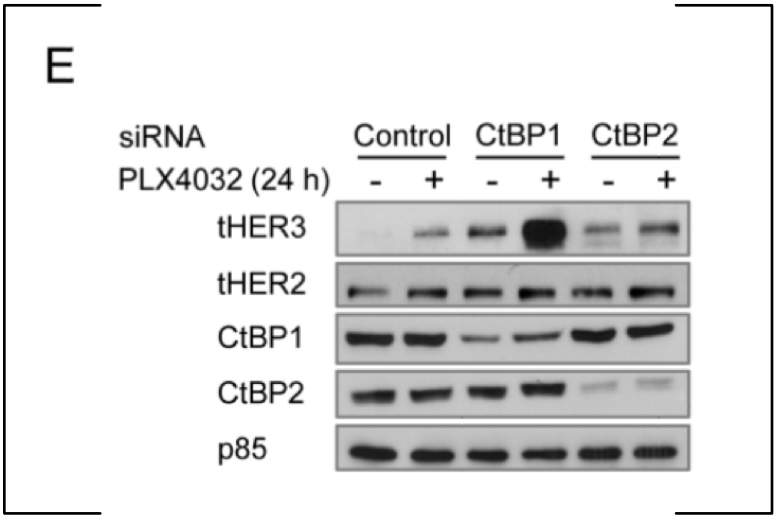
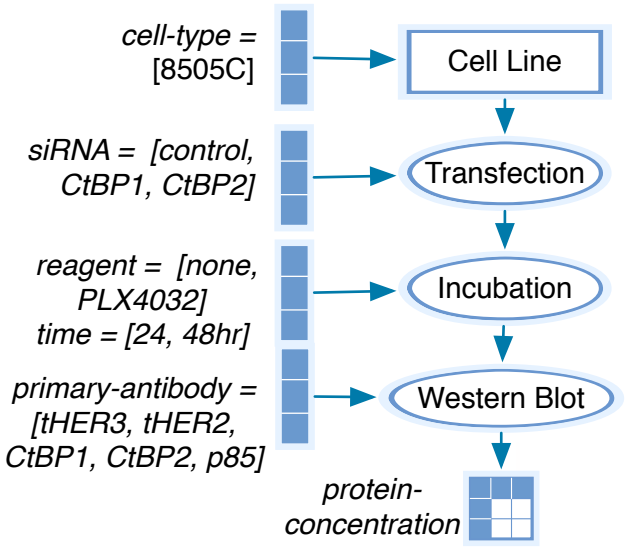


Fig5E Caption

E, panels show protein levels of HER3, HER2, CtBP1, CtBP2 and p85 (loading control) in cells transfected with control, CtBP1 and CtBP2 siRNAs and grown in either absence or presence of 2 μM PLX4032 for 24 h.



This model generates this KEfED data structure:

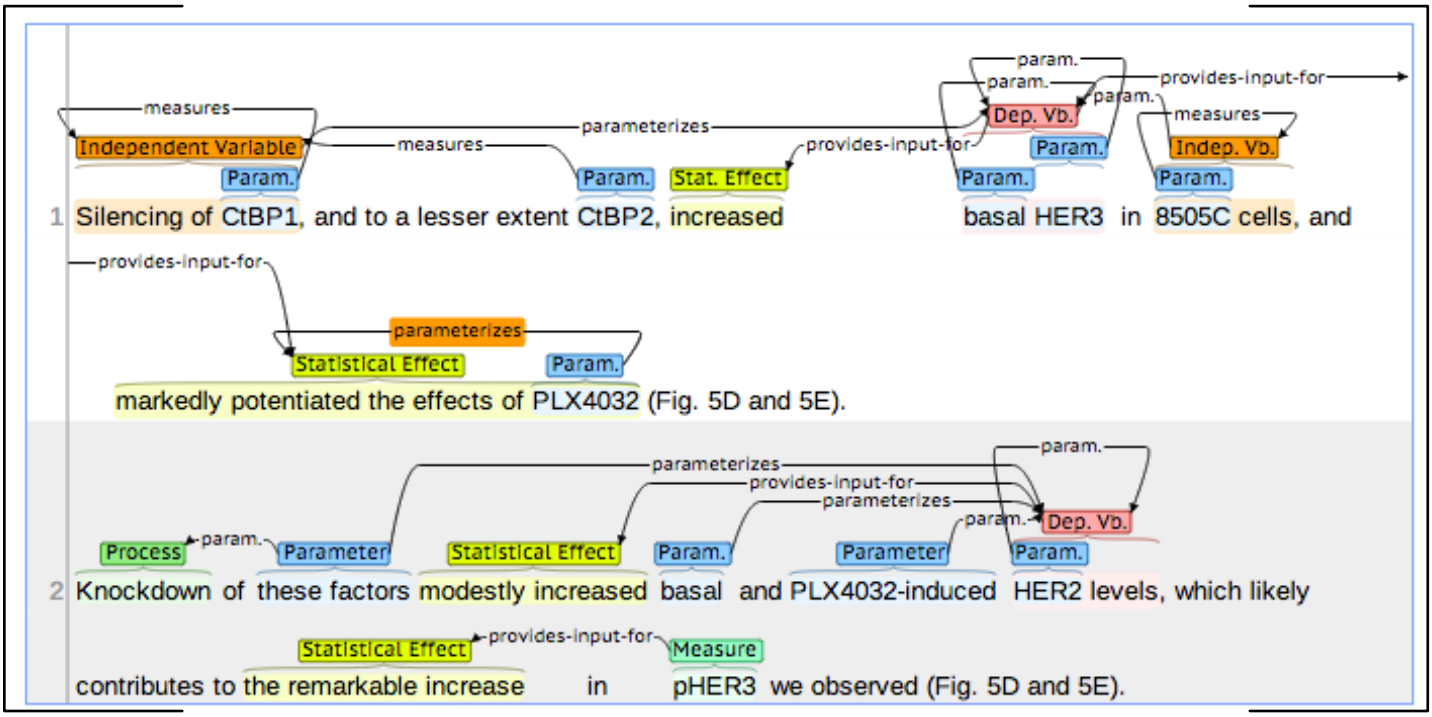
?protein-concentration [?cell-type]
[?siRNA]
[?reagent]
[?time]
[?primary-antibody]

?protein-concentration[8505C][CtBP1]
*[*][24hr][tHER3]*
>> ?protein-concentration[8505C][control]
*[*][24hr][tHER3]*

?protein-concentration[8505C][CtBP1]
*[*][24hr][tHER2]*
> ?protein-concentration[8505C][control]
*[*][24hr][tHER2]*

other elements are controls.

annotation


measures
Independent Variable
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measures
parameterizes
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Stat. Effect
provides-input-for
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param.
Dep. Vb.
Param.
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measures
Indep. Vb.
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1 Silencing of CtBP1, and to a lesser extent CtBP2, increased basal HER3 in 8505C cells, and
provides-input-for
parameterizes
Statistical Effect
Param.
markedly potentiated the effects of PLX4032 (Fig. 5D and 5E).
parameterizes
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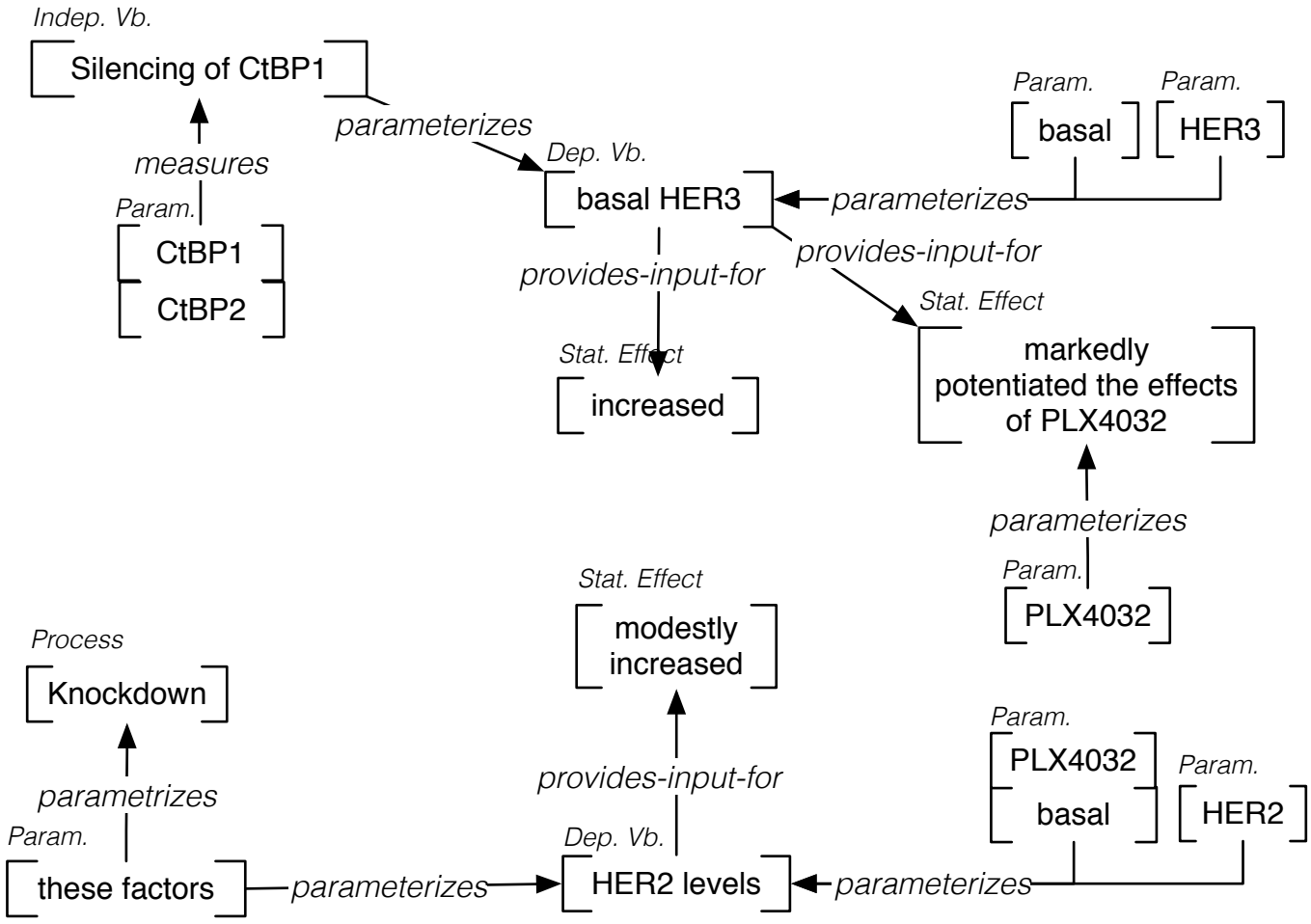
Dep. Vb.
Process
param.
Parameter
Statistical Effect
Param.
Parameter
Param.
2 Knockdown of these factors modestly increased basal and PLX4032-induced HER2 levels, which likely
Statistical Effect
provides-input-for
Measure
contributes to the remarkable increase in pHER3 we observed (Fig. 5D and 5E).


Indep. Vb.
Silencing of CtBP1
measures
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Dep. Vb.
basal HER3
provides-input-for
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markedly potentiated the effects of PLX4032
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basal
Param.
HER2
parameterizes

narrative text

Finally, CtBP1 and CtBP2 chromatin immunoprecipitation assays showed decreased binding to the HER3 promoter after treatment with PLX4032 (Fig. 5F).

Fig5F

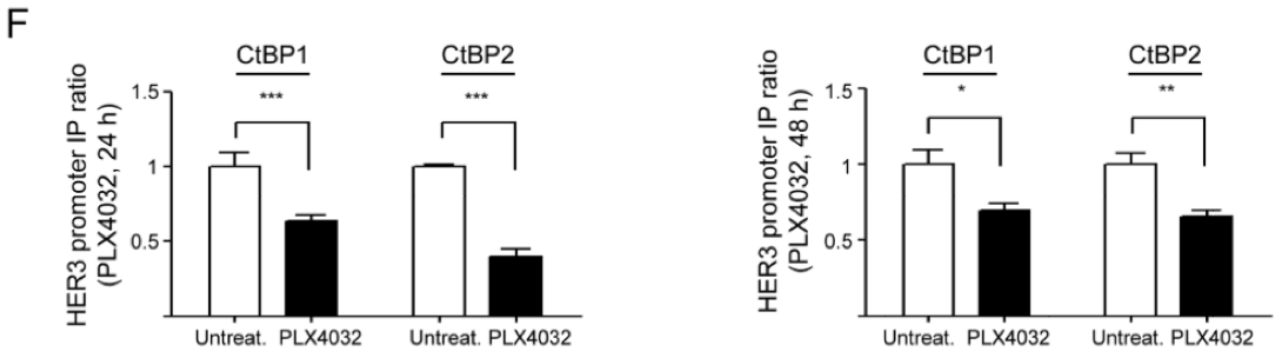
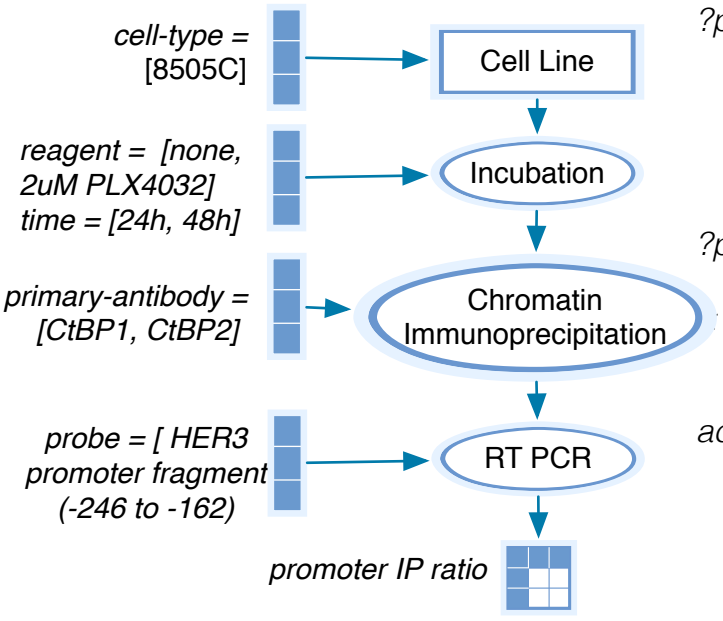


Fig5F Caption

F, CtBP1 and CtBP2 chromatin immunoprecipitation assays were performed in 8505C cells treated with or without 2 μM PLX4032 for 24 (left) or 48 h (right). A fragment (–246 to –162) of the HER3 promoter that includes known interacting sites for CtBP proteins was amplified by means of RT-PCR for both conditions. Graph shows normalized RT-PCR data of HER3 fragment of the immunoprecipitated complex compared to input lysate. Data represent mean +/– SD of two independent biological replicates performed in quadruplicate



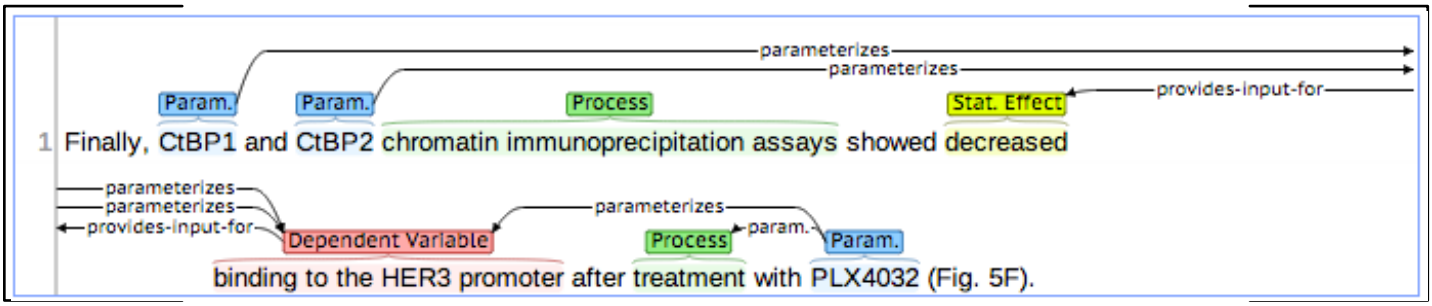
This model generates this KEfED data structure:

?promoter-ip-ratio [?cell-type]
[?reagent]
[?time]
[?primary-antibody]
[?probe]

?promoter-ip-ratio [8505C][PLX4032]
*[*][*][HER3 frg]*
?promoter-ip-ratio [8505C][none]
*[*][*][HER3 frg]*

across all values of ?time and ?primary-antibody

annotation


parameterizes
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Stat. Effect
1 Finally, CtBP1 and CtBP2 chromatin immunoprecipitation assays showed decreased
parameterizes
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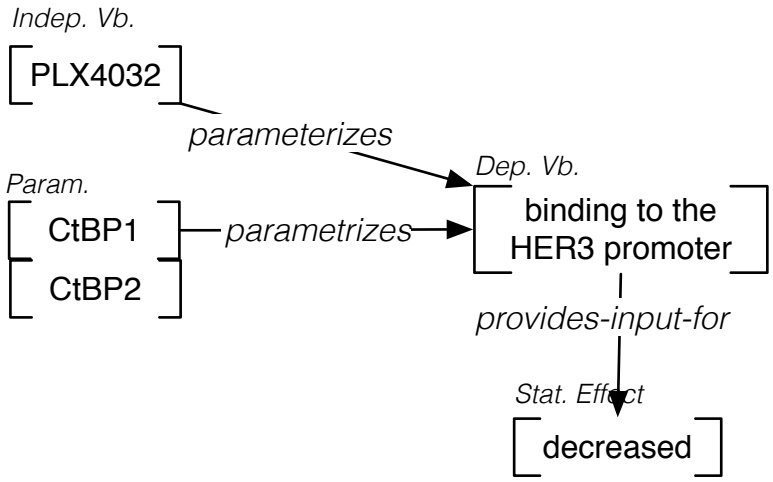
Dependent Variable
parameterizes
Process
param.
Param.
binding to the HER3 promoter after treatment with PLX4032 (Fig. 5F).


Indep. Vb.
PLX4032
parameterizes
Dep. Vb.
Param.
CtBP1
parametrizes
binding to the HER3 promoter
CtBP2
provides-input-for
Stat. Effect
decreased