Averaging of biological replicates

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Contents

1 Averaging all bio replicates of PIP3

1.1 Separated siINPP4(A+B) and siSHIP2 conditions

Looking at the file bio-reps-summary.pdf I decided to normalize all PIP3 data to the peak value in PTEN-sictrl, which is about 0.01 (in Golden Ration units). To do this I merge all data from the following files into one file and treat these different data as biological replicates:

2014_09_05B_c4_FULLn1

2014_09_19B_c4_FULLn2

2015_02_23B_c4_FULLn3

2014_12_18A_c4_full

2015_02_06B_c4_pten_ship2_ptenship2_n2

2015_04_04A_c4_wt_ship2_pten_ptenship2_N3

2014_12_12A_c4_wt_ab_pten_tc

2015_02_14B_c4_pten_ab_pab_tcN2_pabVSpabsN2

2015_03_06A_c4_pi34p2Trans_n3

2015_08_06A_c4_PTEN_crispr_n1

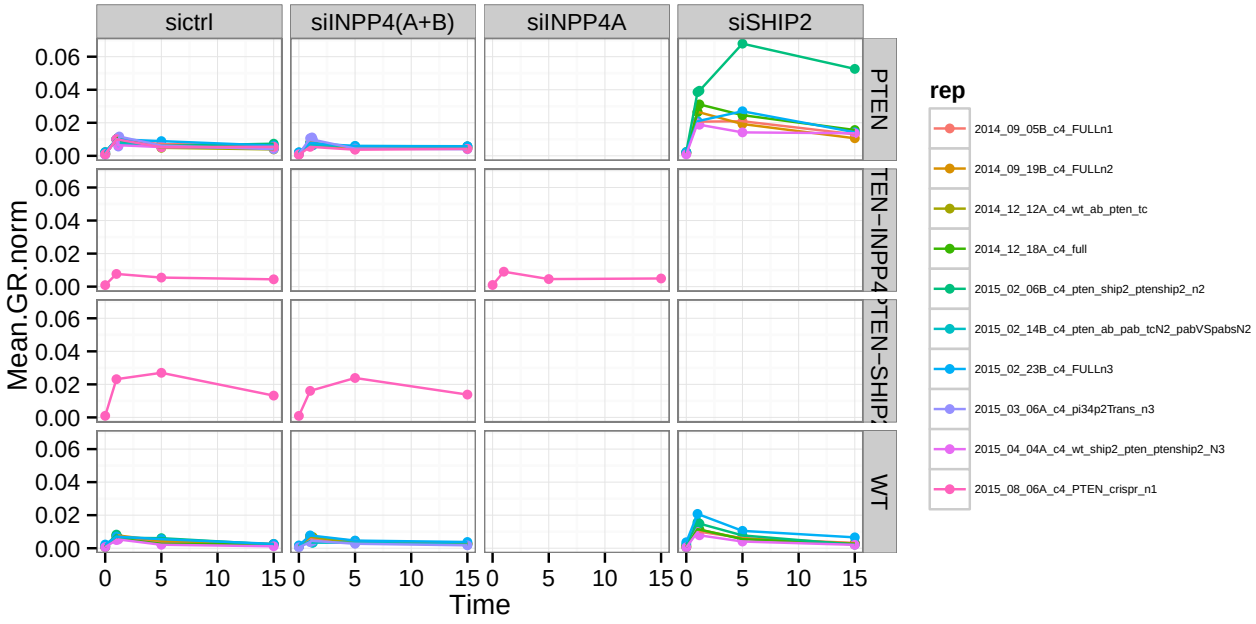
Then I find a coefficient of normalisation for each replicate (so that it should be always 0.01 in PTEN-sictrl condition)

rep	norm.coef
2014_09_05B_c4_FULLn1	0.743657678035968
2014_09_19B_c4_FULLn2	0.917853550380810

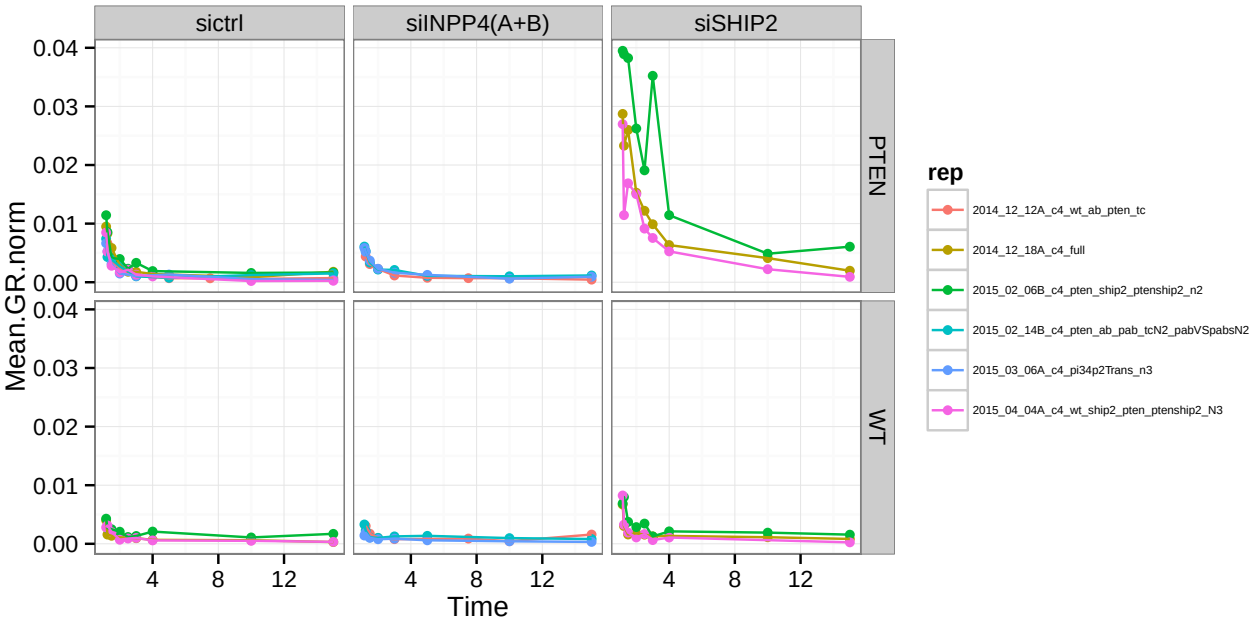
rep	norm.coef
2015_02_23B_c4_FULLn3	3.876077770904362
2014_12_18A_c4_full	0.948582326607152
2015_02_06B_c4_pten_ship2_ptenship2_n2	2.572207144731321
2015_04_04A_c4_wt_ship2_pten_ptenship2_N3	3.126230418069979
2014_12_12A_c4_wt_ab_pten_tc	1.057647838786254
2015_02_14B_c4_pten_ab_pab_tcN2_pabVSpabsN2	1.461183579234045
2015_03_06A_c4_pi34p2Trans_n3	3.511431787232272
2015_08_06A_c4_PTEN_crispr_n1	0.842264131853826

Then I normalise each biological replicate by its coefficient.

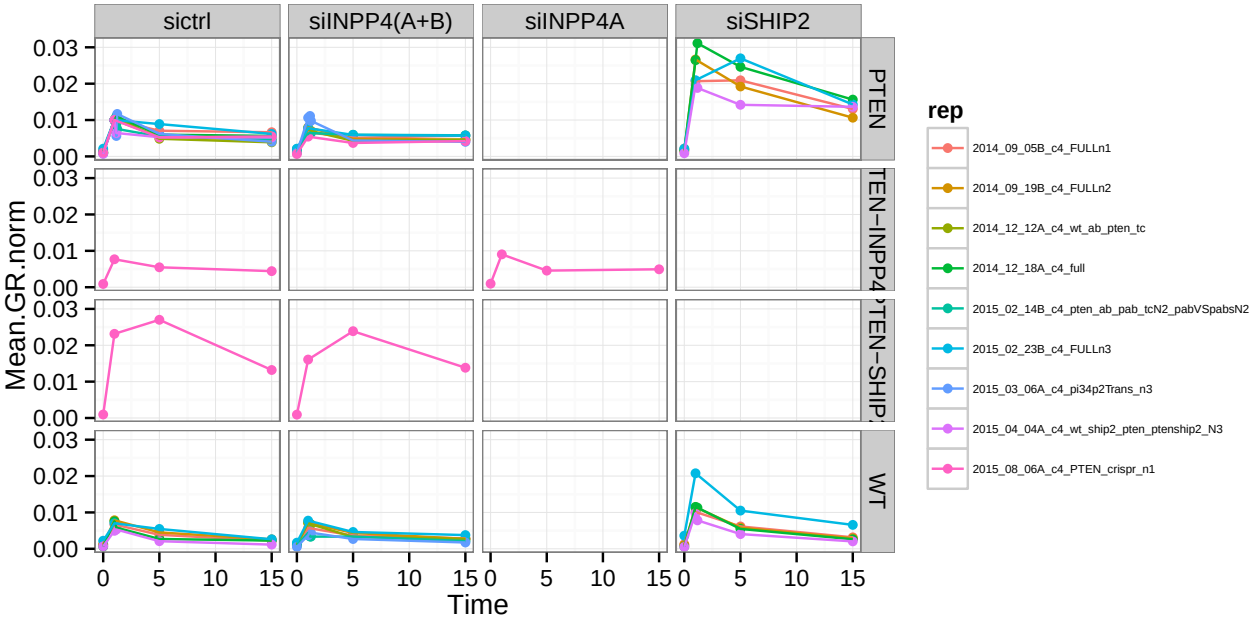
The resulting plot for PIP3 without PI3K inhibitor:

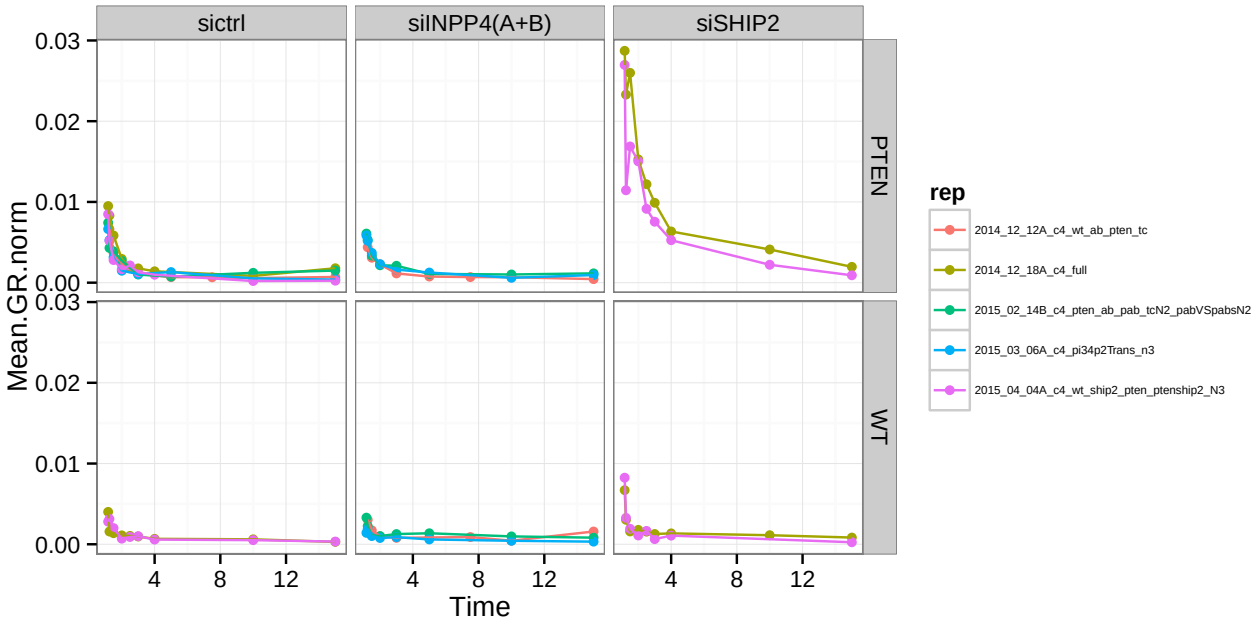


The resulting plot for PIP3 with PI3K inhibitor:

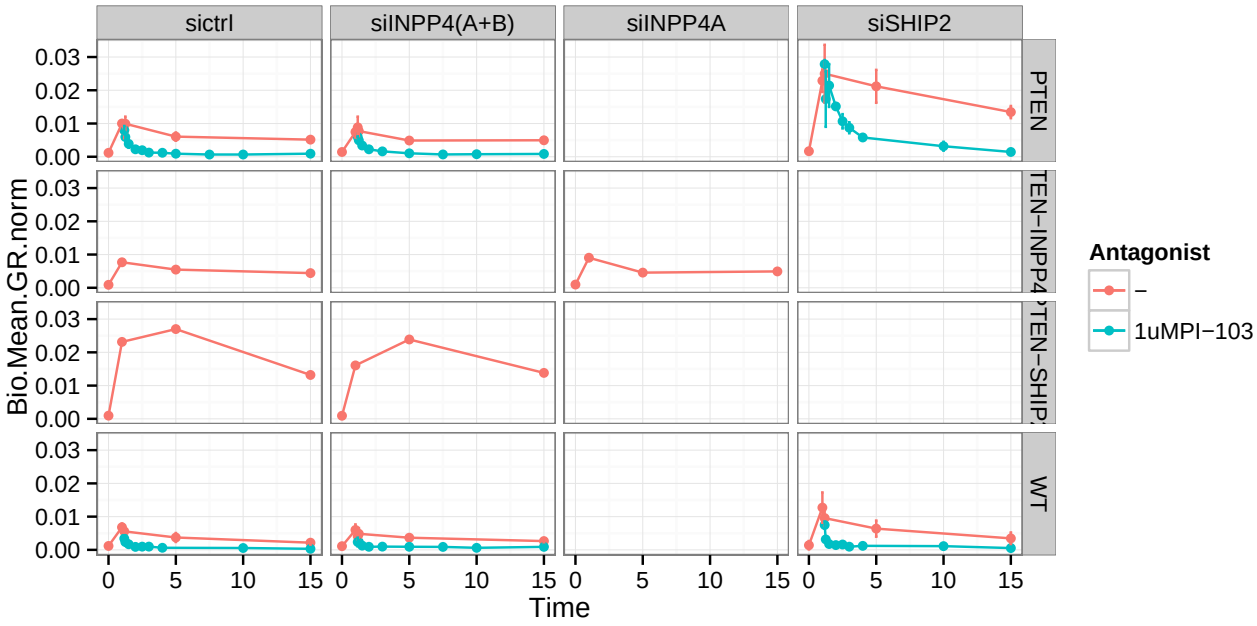


Now I remove the outlier experiment 2015_02_06B_c4_pten_ship2_ptenship2_n2 (it is an outlier for both cases with and without PI3K inhibitor). The resulting plots are the following:





Now I will average all of the replicates shown in the previous figure and plot the averaged data with error bars corresponding to biological noise:



1.2 siINPP4(A+B)&siSHIP2 condition

Note that there are still two experiments left in which we looked at double silencing of INPP4(A+B) and SHIP2:

2014_10_10A_c4_pabVSpabs

2015_02_14B_c4_pabVSpabsN2

I have not used them in the previous section because they do not have PTEN-sictrl condition and would not be able to normalise them. But now, looking the last figure, I can normalise them by PTEN-siINPP4(A+B)

condition (all other bio replicates are quite consistent in this point). The PIP3 concentration at this point should be:

```
d1[Genotype == "PTEN" & Condition == "siINPP4(A+B)" & Time == 1, Bio.Mean.GR.norm]
```

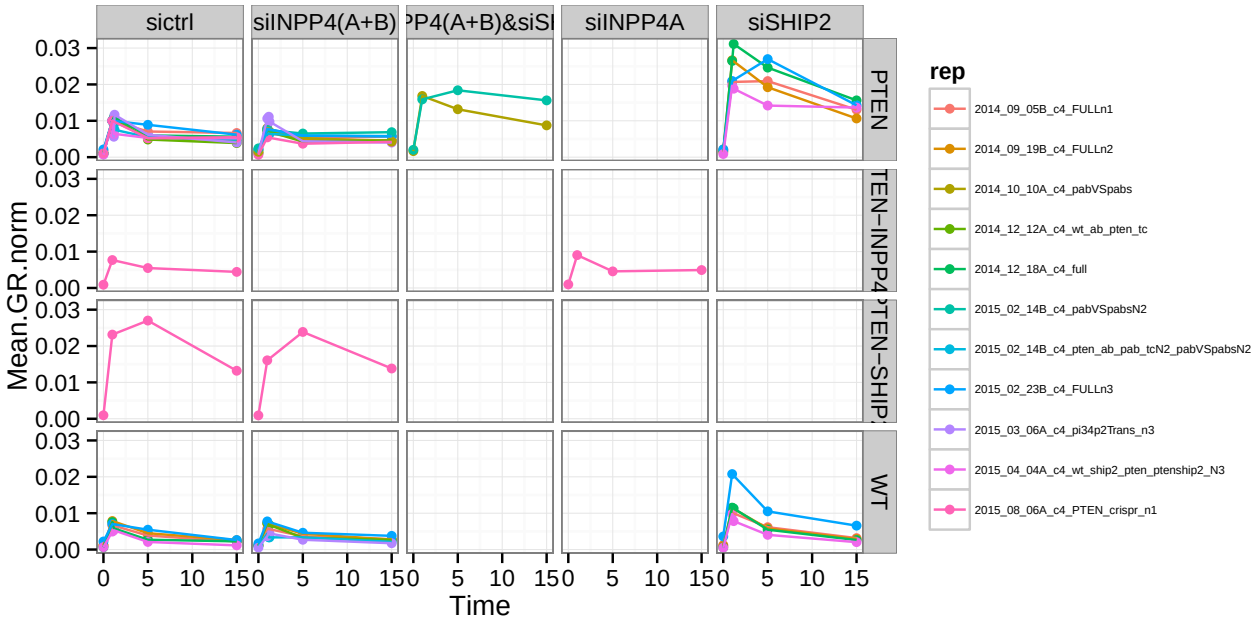
```
## [1] 0.00745414134784178
```

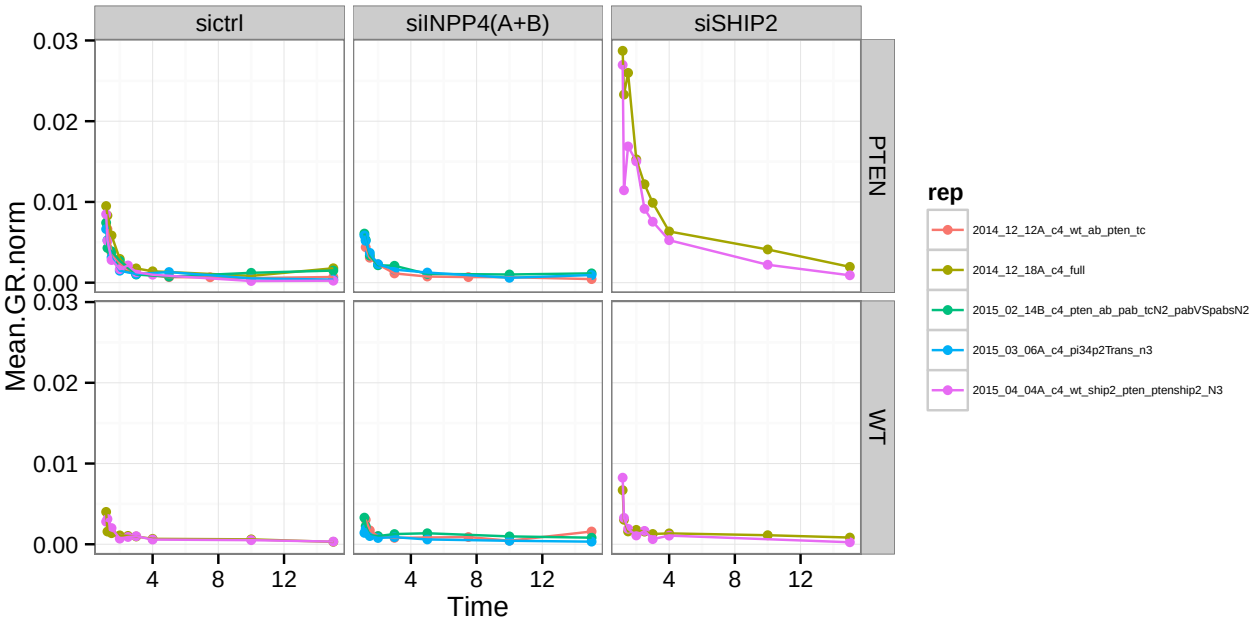
So, first I normalise these two experiments by this time point:

rep	norm.coef
2014_10_10A_c4_pabVSpabs	0.716750190606507
2015_02_14B_c4_pabVSpabsN2	1.784728351457595

And then merge these two replicates with the ones defined before.

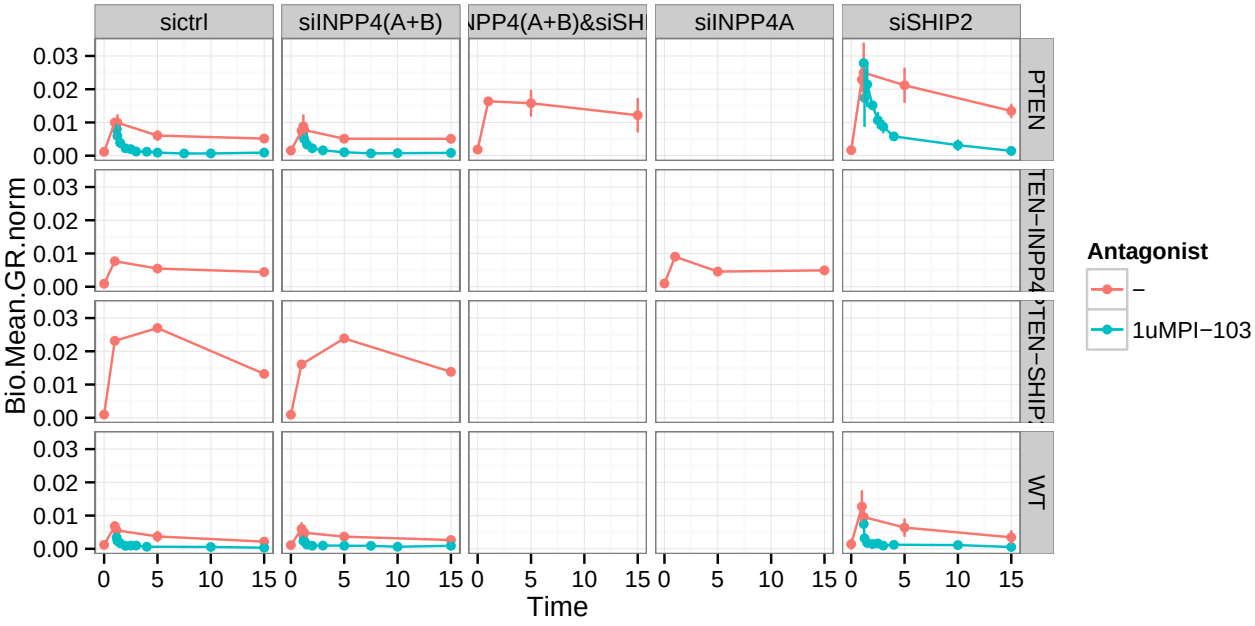
Now the total data set looks like:





1.3 Final plot of PIP3 data

And averaging of all biological replicates provides a final plot containing all PIP3 data (I will be working with it in the modeling part):



2 Averaging all bio replicates of PI(3,4)P2

The same procedure as in the previous chapter can be done for PI(3,4)P2 lipids.

2.1 Separated siINPP4(A+B) and siSHIP2 conditions

Looking at the file bio-reps-summary.pdf I decided to normalize all PI(3,4)P2 data to the peak value in PTEN-sictrl. To do this I merge all data from the following files into one file and treat these different data as biological replicates:

2014_09_05A_c18_FULLn1

2014_09_19A_c18_FULLn2

2015_02_23A_c18_FULLn3

2014_12_12B_c18_wt_ab_pten_tc

2015_02_14A_c18_pten_ab_pab_tcN2_pabVSpabsN2

2015_03_06B_c18_pi34p2Trans_n3

2015_08_06B_c18_PTEN_crispr_n1

To find the averaged concentration in PTEN-sictrl condition I simply average the data in this point (there are no really bad outliers in PI(3,4)P2 data, so it is possible to do it):

```
d[Time == 1 & Condition == "sictrl" & Genotype == "PTEN", list(mean(Mean.GR))]
```

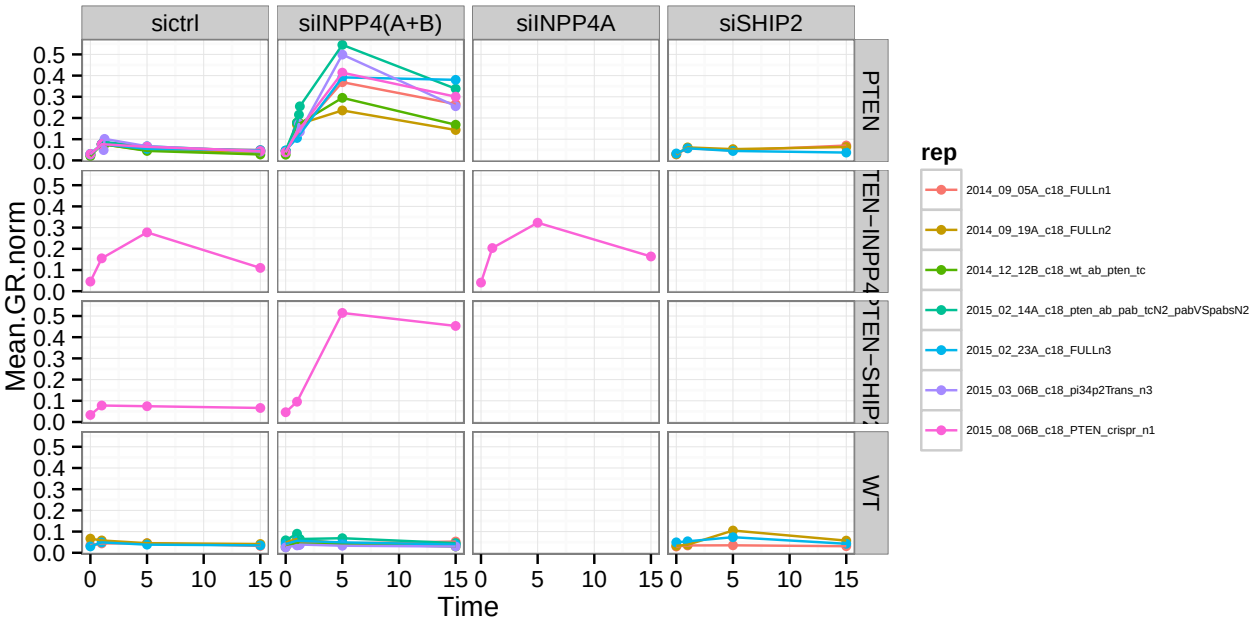
```
##                     V1
## 1: 0.0765314027687824
```

Then I find a coefficient of normalisation for each replicate (so that it should be always 0.0765314027687824 in PTEN-sictrl condition)

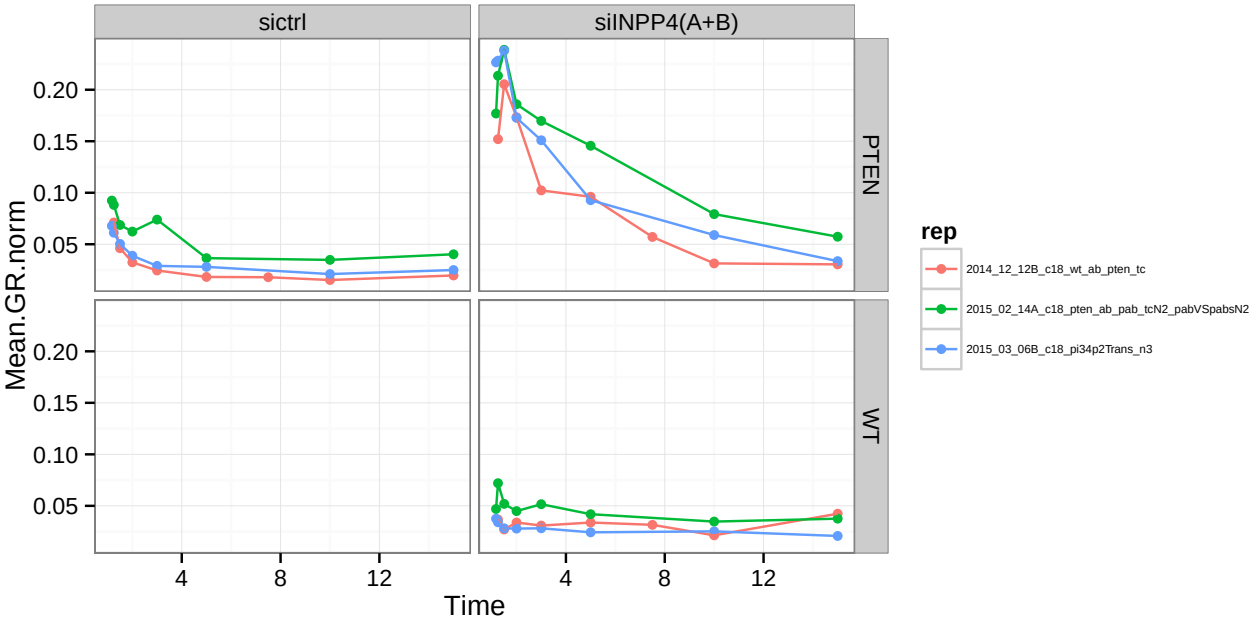
rep	norm.coef
2014_09_05A_c18_FULLn1	1.136474167778717
2014_09_19A_c18_FULLn2	1.060293486809454
2015_02_23A_c18_FULLn3	1.066929539741907
2014_12_12B_c18_wt_ab_pten_tc	0.887924335445865
2015_02_14A_c18_pten_ab_pab_tcN2_pabVSpabsN2	1.261817988105321
2015_03_06B_c18_pi34p2Trans_n3	0.693827082989926
2015_08_06B_c18_PTEN_crispr_n1	1.136788951176873

Then I normalise each biological replicate by its coefficient.

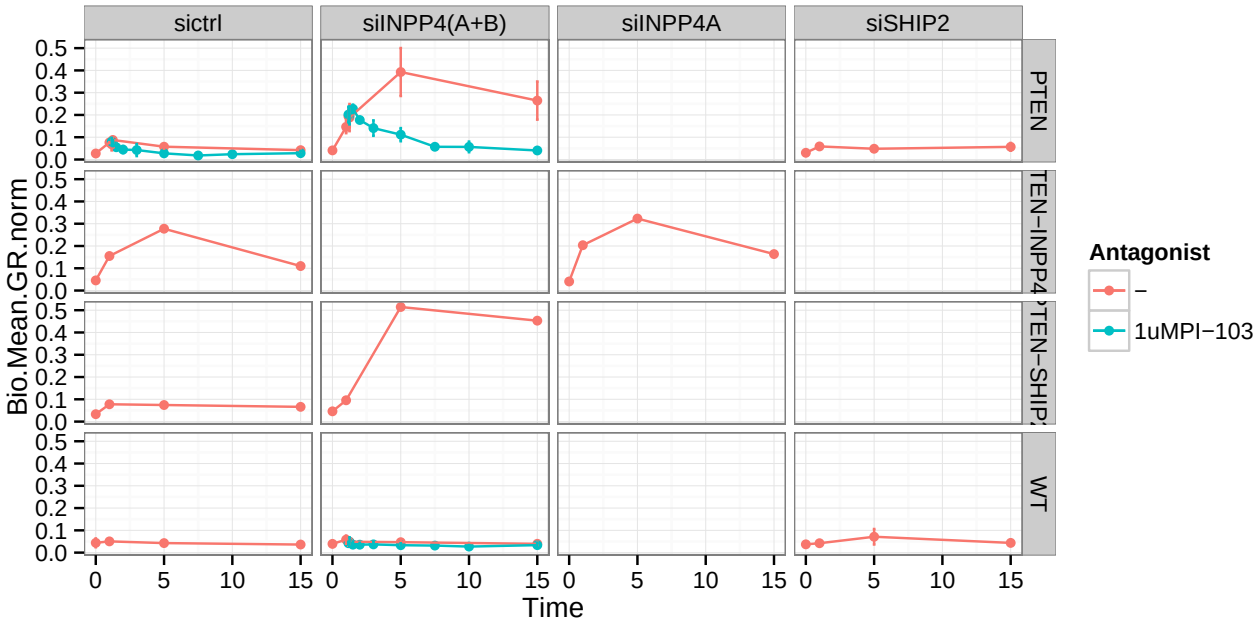
The resulting plot for PIP3 without PI3K inhibitor:



The resulting plot for PIP3 with PI3K inhibitor:



Now I will average all of the replicates shown in the previous figure and plot the averaged data with error bars corresponding to biological noise:



2.2 siINPP4(A+B)&siSHIP2 condition

Note that there are still two experiments left in which we looked at double silencing of INPP4(A+B) and SHIP2:

2014_10_10B_c18_pabVSpabs

2015_02_14A_c18_pabVSpabsN2

I have not used them in the previous section because they do not have PTEN-sictrl condition and would not be able to normalise them. But now, looking the last figure, I can normalise them by PTEN-siINPP4(A+B) condition (all other bio replicates are quite consistent in this point). The PIP3 concentration at this point should be:

```
d1[Genotype == "PTEN" & Condition == "siINPP4(A+B)" & Time == 1, Bio.Mean.GR.norm]
```

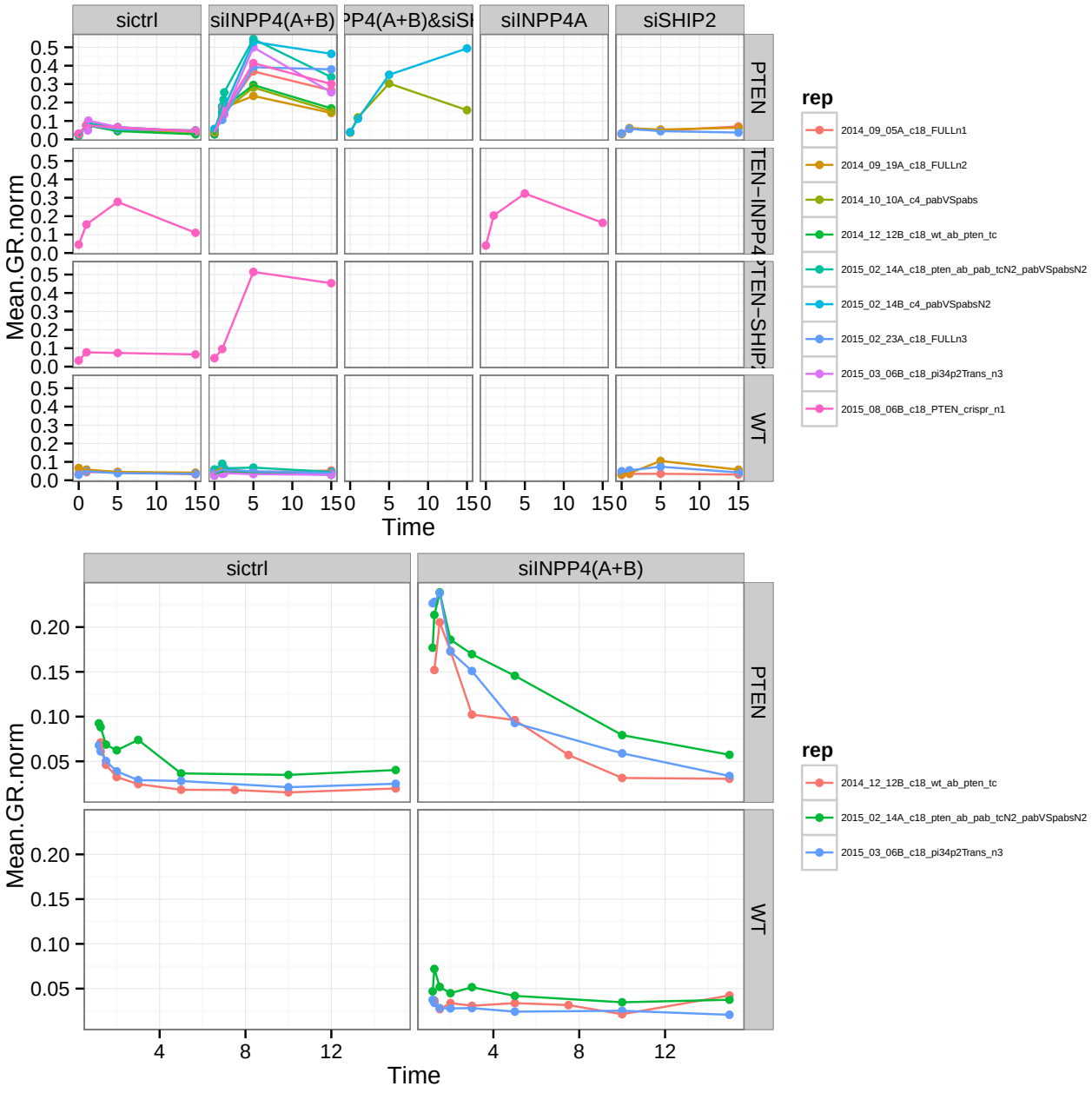
```
## [1] 0.146433426237419
```

So, first I normalise these two experiments by this time point:

rep	norm.coef
2014_10_10A_c4_pabVSpabs	0.526359540998913
2015_02_14B_c4_pabVSpabsN2	1.267417053639766

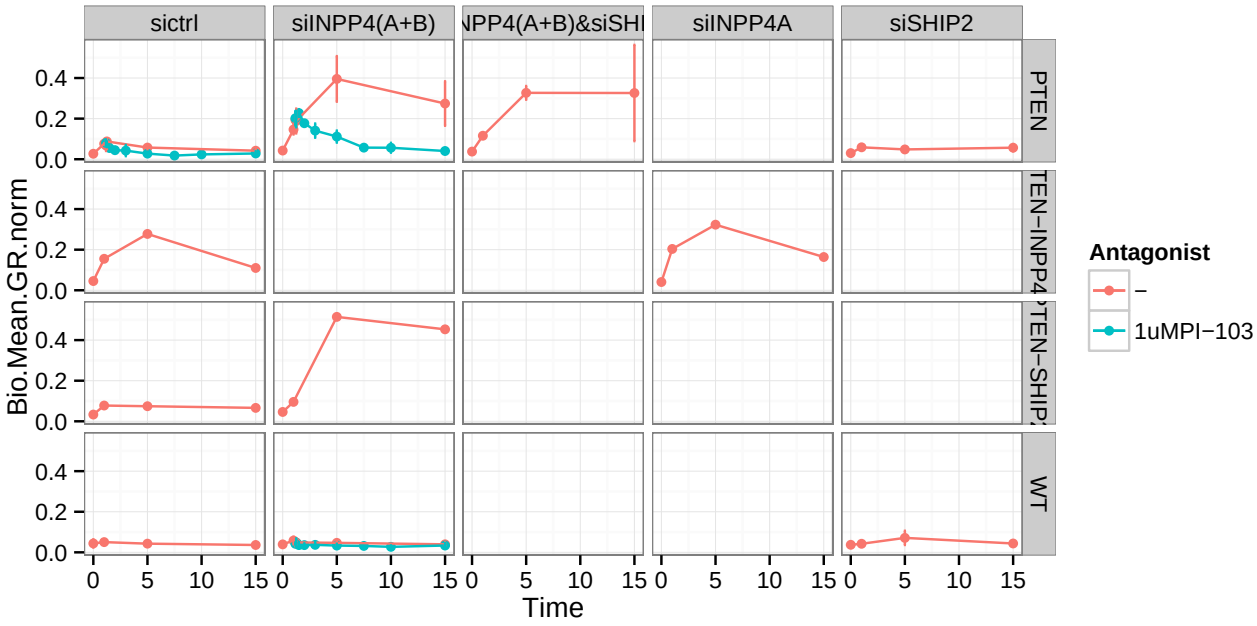
And then merge these two replicates with the ones defined before.

Now the total data set looks like:



2.3 Final plot of PI(3,4)P2 data

And averaging of all biological replicates provides a final plot containing all PI(3,4)P2 data (I will be working with it in the modeling part):


sictrl
siINPP4(A+B)
NPP4(A+B)&siSHI
siINPP4A
siSHIP2
PTEN
TEN−INPP4
TEN−SHIP
WT
Bio.Mean.GR.norm
0.0
0.2
0.4
0
5
10
15
Time
Antagonist
–
1uMPI−103