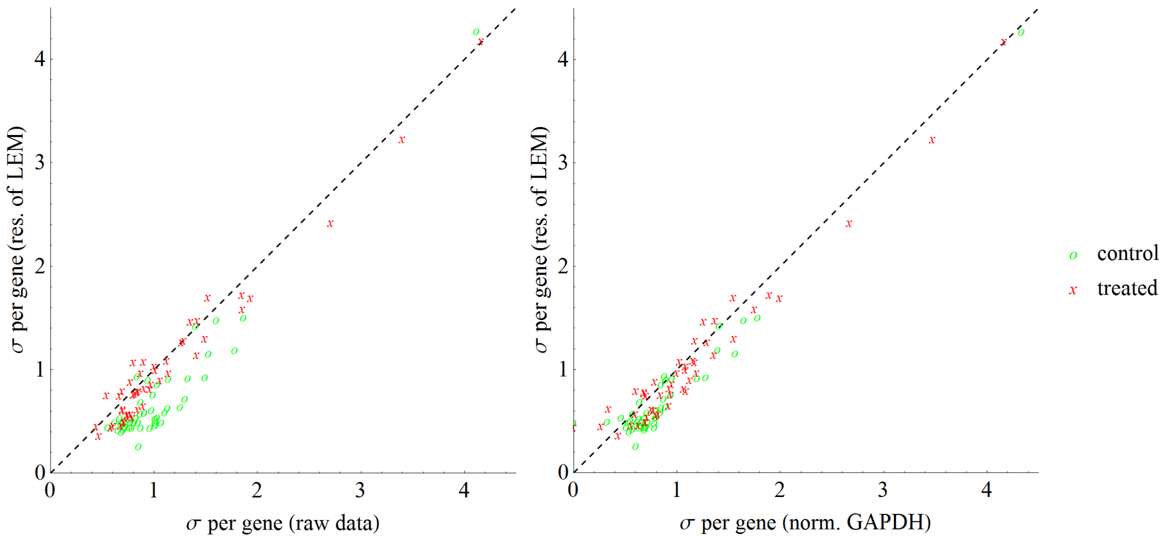


Figure 1: Comparision of standard deviation σ per gene for data set 1. Left: Raw data versus LEMming. Right: $\Delta\Delta CT$ normalized data (reference gene: *Gapdh*) versus LEMming processed data. Both cases show that σ's per gene of LEMming processed are smaller.

Error variance of $\Delta\Delta C_t$ method in comparison with LEMming

Here, we want to proof, that LEMming results in a decreased standard deviation compared $\Delta\Delta C_t$ with one reference gene (RG). In the following we use abbreviations for *ref* - r, *target* - t, *sample* - s and *control* - c. For sake of simplicity we make three simplifying assumptions: We assume that the PCR efficiency $E_t = E_r$, the $\Delta\Delta C_t$ is

$$\Delta\Delta C_t = (C_t^{r,s} - C_t^{t,s}) - (C_t^{r,c} - C_t^{t,c})$$

We assume that control and sample measurements originate from different animals. According to LEMming the C_t-values can be decomposed (e.g. $C_t^{r,s} = \epsilon_{P:A,r} + \epsilon_{S,s} + \Delta_{T,s} + \Delta_{T:G,r,s} + \epsilon_{r,s}$). Calculating the $\Delta\Delta C_t$ value the probe and sample errors cancel out. The variables $\Delta_{T,\_}$ and $\Delta_{T:G,\_,\_}$ with the according indices are not classified as random variables. Thus, their variance is zero. The random variables $\epsilon_{r,s}$, $\epsilon_{r,c}$, $\epsilon_{t,s}$ and $\epsilon_{t,c}$ are independently distributed.

$$Var(\Delta\Delta C_t) = Var(\epsilon_{r,s} - \epsilon_{t,s} - \epsilon_{r,c} + \epsilon_{t,c})$$

Assuming that all ϵ's have the same variance σ_ϵ^2, the variance of $\Delta\Delta C_t$ is given by $4\sigma_\epsilon^2$.
The ΔC_t (distance to the mean of the control) of LEMming has the variance

$$Var(\Delta C_t) = Var(\epsilon_{t,s}) + Var(Mean(\epsilon_{t,c}))$$

With all ϵ's being equal the variance is of the ΔC_t is $\sigma_\epsilon^2 + \frac{\sigma_\epsilon^2}{n}$ where n is the size of the control group. This means that $Var(\Delta C_t) < Var(\Delta\Delta C_t)$, proving that LEMming has lower variance compared to the $\Delta\Delta C_t$ method. If the variances of control group and treated group are different, as we have shown for DS1 in the main manuscript, only $Var(\epsilon_{t,s})$ cancel out and the variances are:

$$Var(Mean(\epsilon_{t,c})) < Var(\epsilon_{r,s}) + Var(\epsilon_{r,c}) + Var(\epsilon_{t,c}).$$

If sample and control are from the same animals, the random variables ϵ are not statistically independent. The covariance of ϵ's can reduce the variances of the $\Delta\Delta C_t$ method.

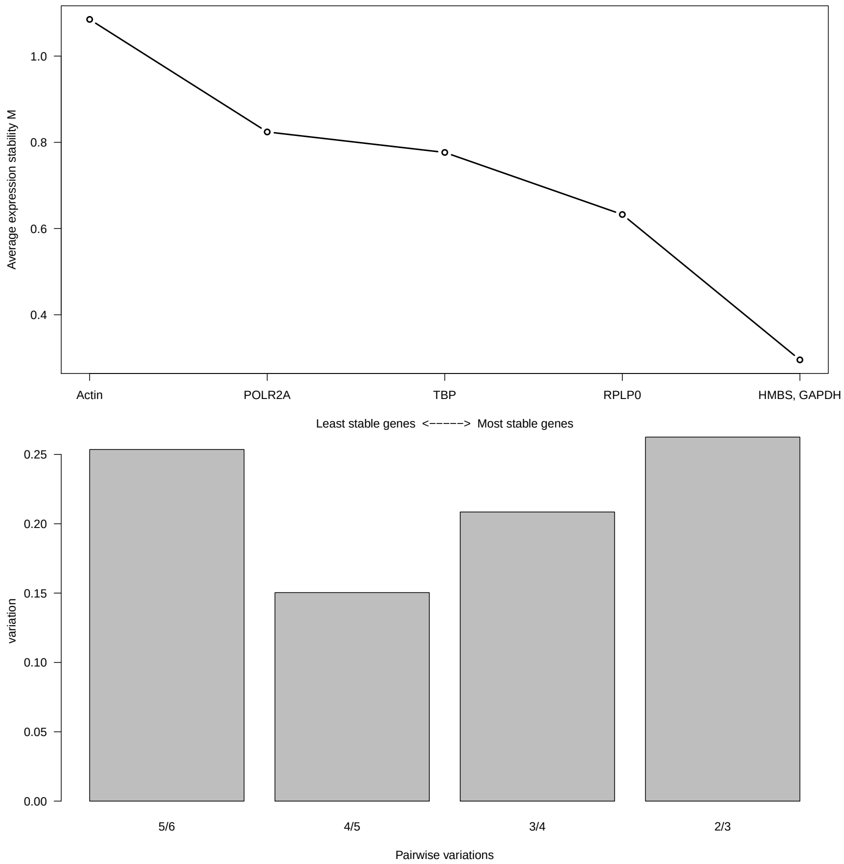
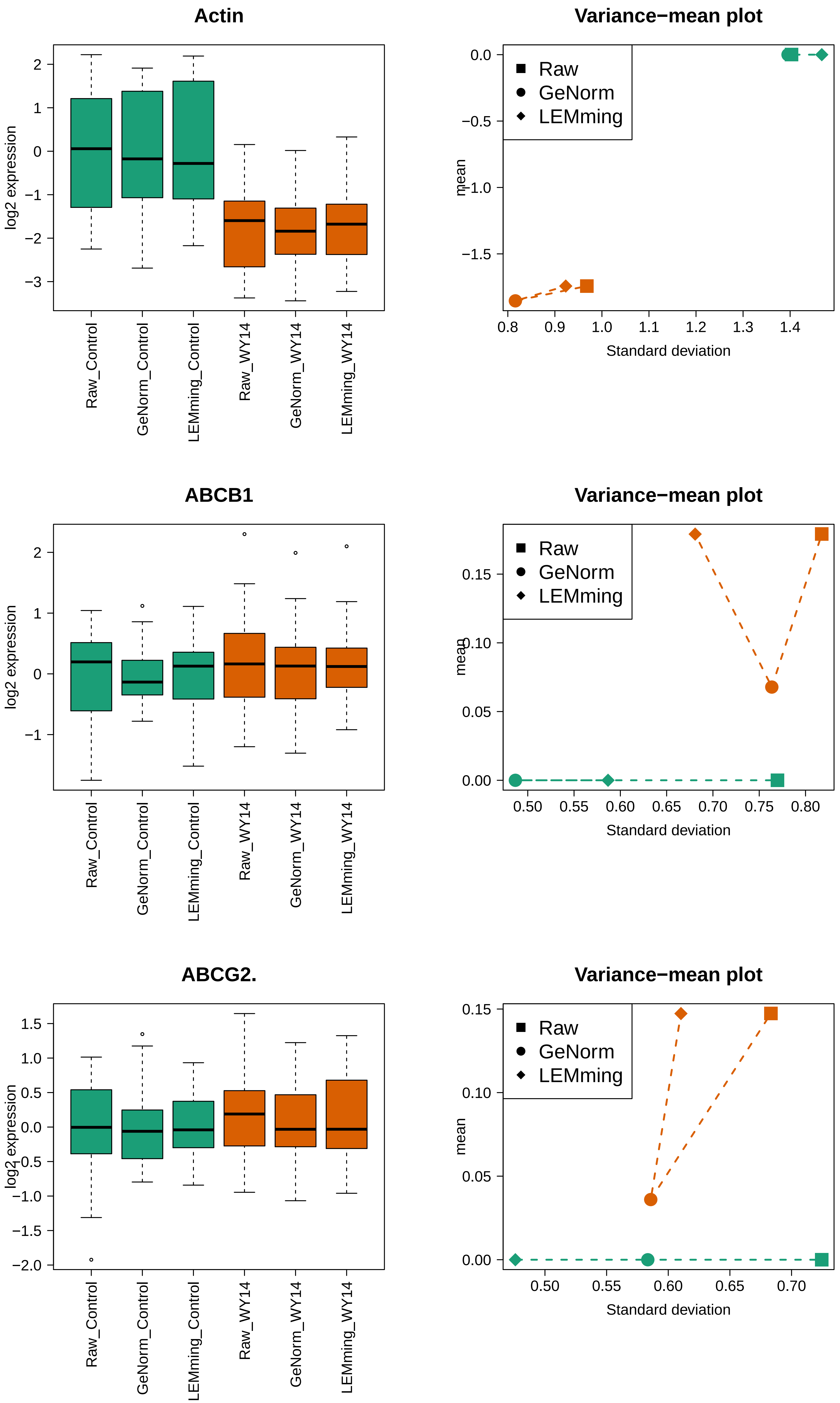


Figure 2: Average expression stability and pairwise variation of data set 1 according to Vandesompele et al. 2002. We selected *GAPDH, HMBS, RPLP0* and *TBP* with a pairwise variation of 0.15 and M-values below 0.8 as RGs for *geNorm* normalization.



Actin
log2 expression
2
1
0
−1
−2
−3
Raw_Control
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
0.0
−0.5
−1.0
−1.5
0.8
0.9
1.0
1.1
1.2
1.3
1.4
Standard deviation
ABCB1
log2 expression
2
1
0
−1
Raw_Control
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
0.15
0.10
0.05
0.00
0.50
0.55
0.60
0.65
0.70
0.75
0.80
Standard deviation
ABCG2.
log2 expression
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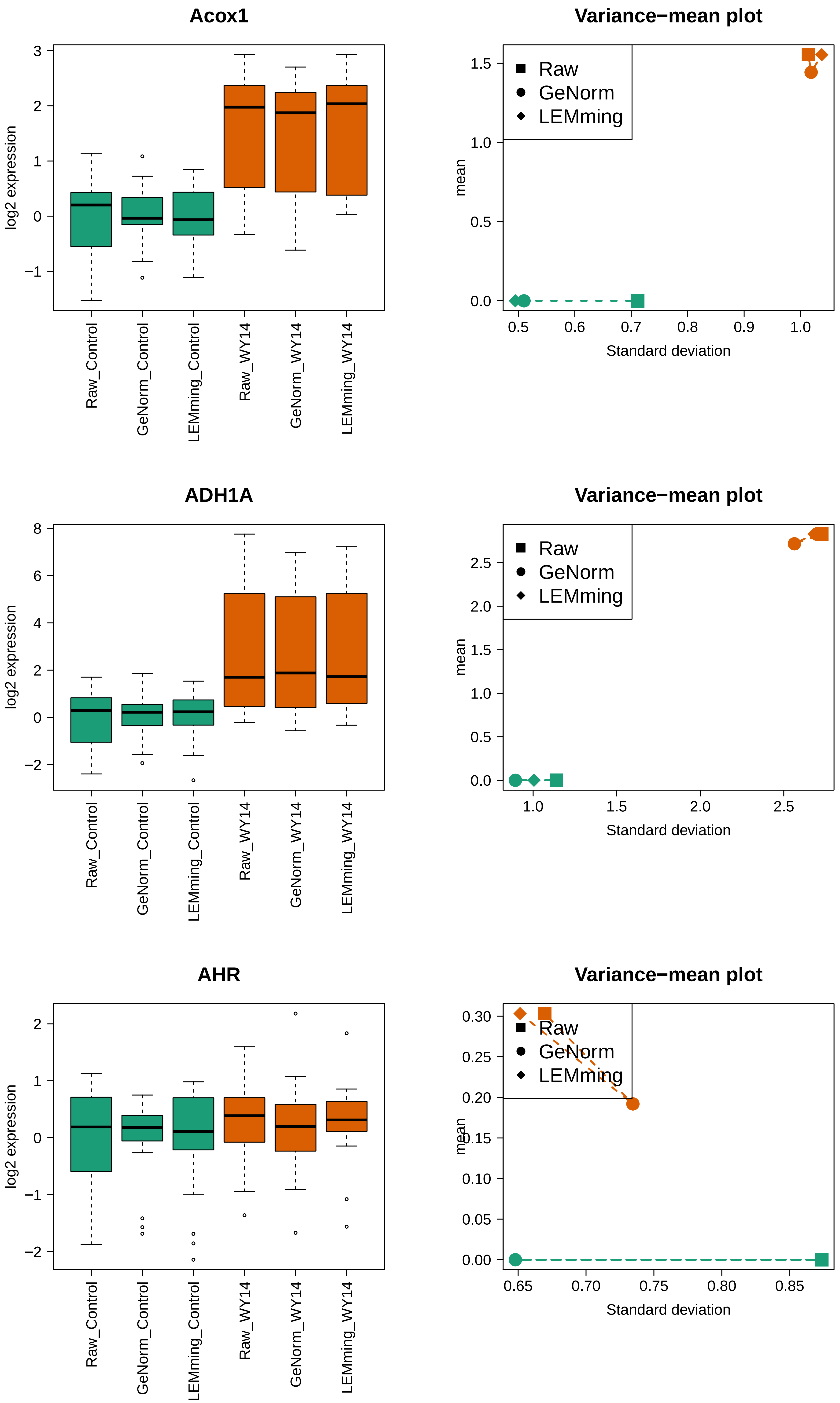
0.5
0.0
−0.5
−1.0
−1.5
−2.0
Raw_Control
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
0.15
0.10
0.05
0.00
0.50
0.55
0.60
0.65
0.70
Standard deviation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GeNorm
LEMming
mean
Standard deviation
ADH1A
log2 expression
Raw_Control
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
Standard deviation
AHR
log2 expression
Raw_Control
GeNorm_Control
LEMming_Control
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GeNorm_WY14
LEMming_WY14
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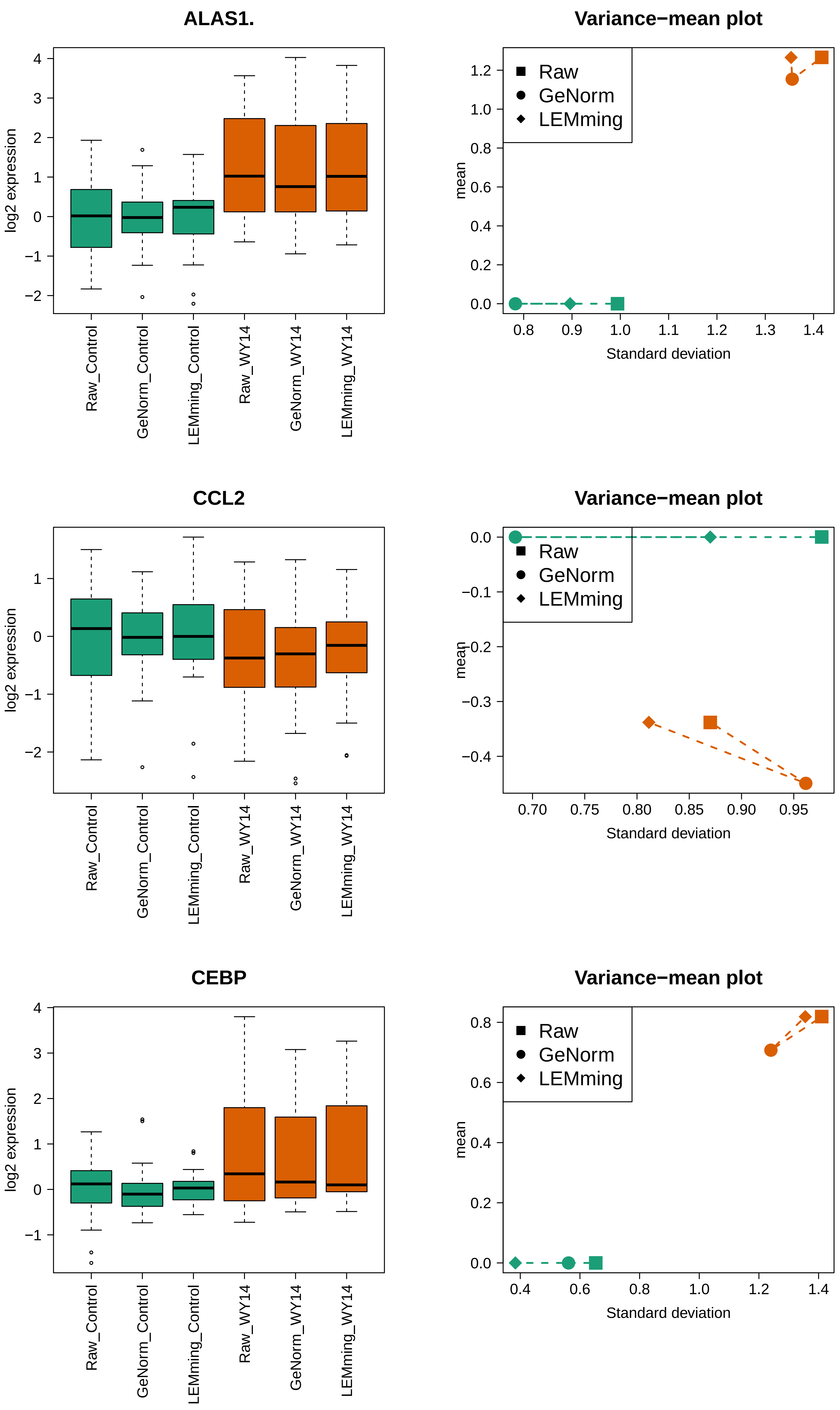
GeNorm
LEMming
mean
Standard deviation



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LEMming_Control
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GeNorm_WY14
LEMming_WY14
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GeNorm
LEMming
mean
Standard deviation
CCL2
log2 expression
Raw_Control
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
Standard deviation
CEBP
log2 expression
Raw_Control
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GeNorm_WY14
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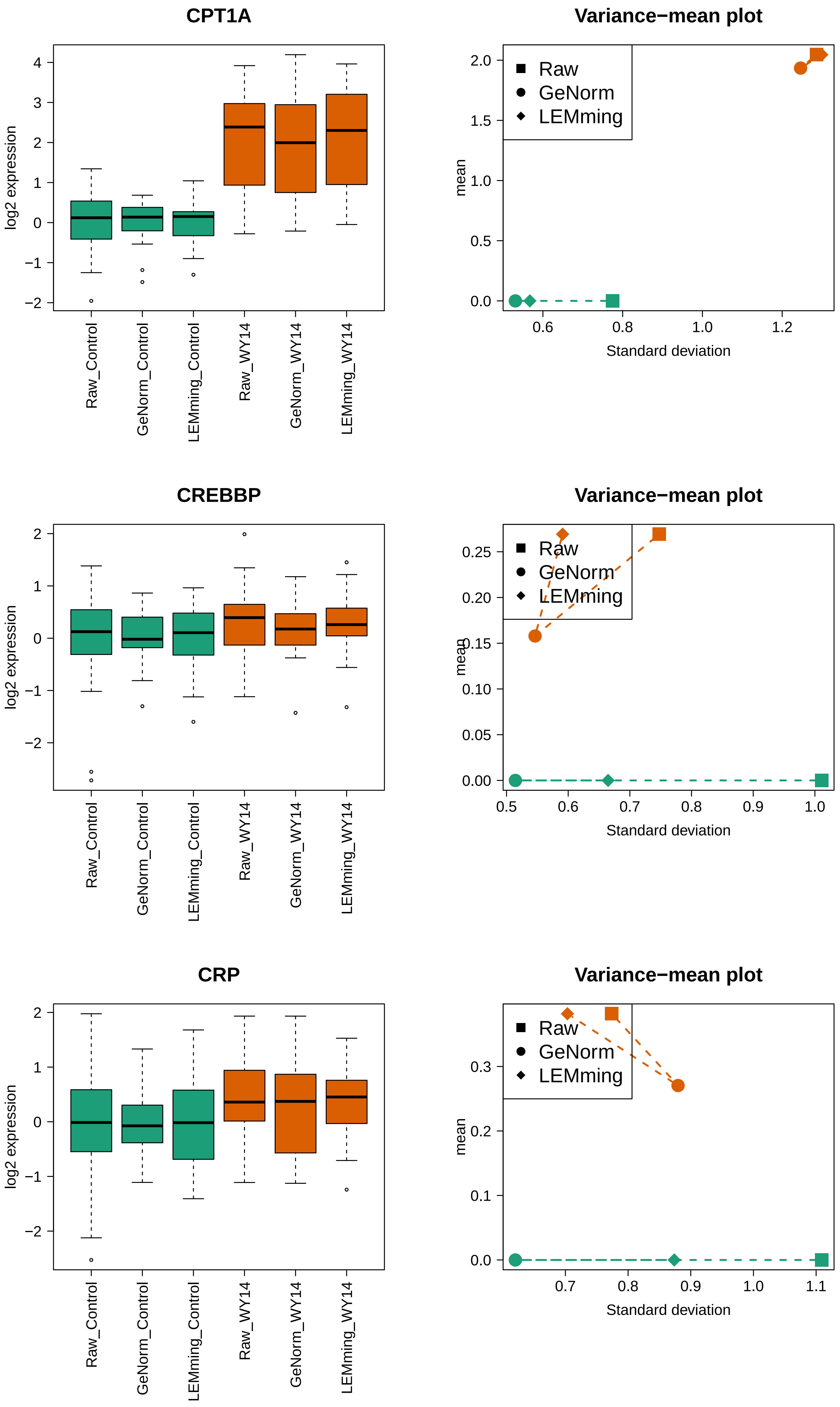
Raw
GeNorm
LEMming
mean
Standard deviation



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LEMming_Control
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GeNorm_WY14
LEMming_WY14
Variance−mean plot
mean
Standard deviation
Raw
GeNorm
LEMming
CREBBP
log2 expression
Raw_Control
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
mean
Standard deviation
Raw
GeNorm
LEMming
CRP
log2 expression
Raw_Control
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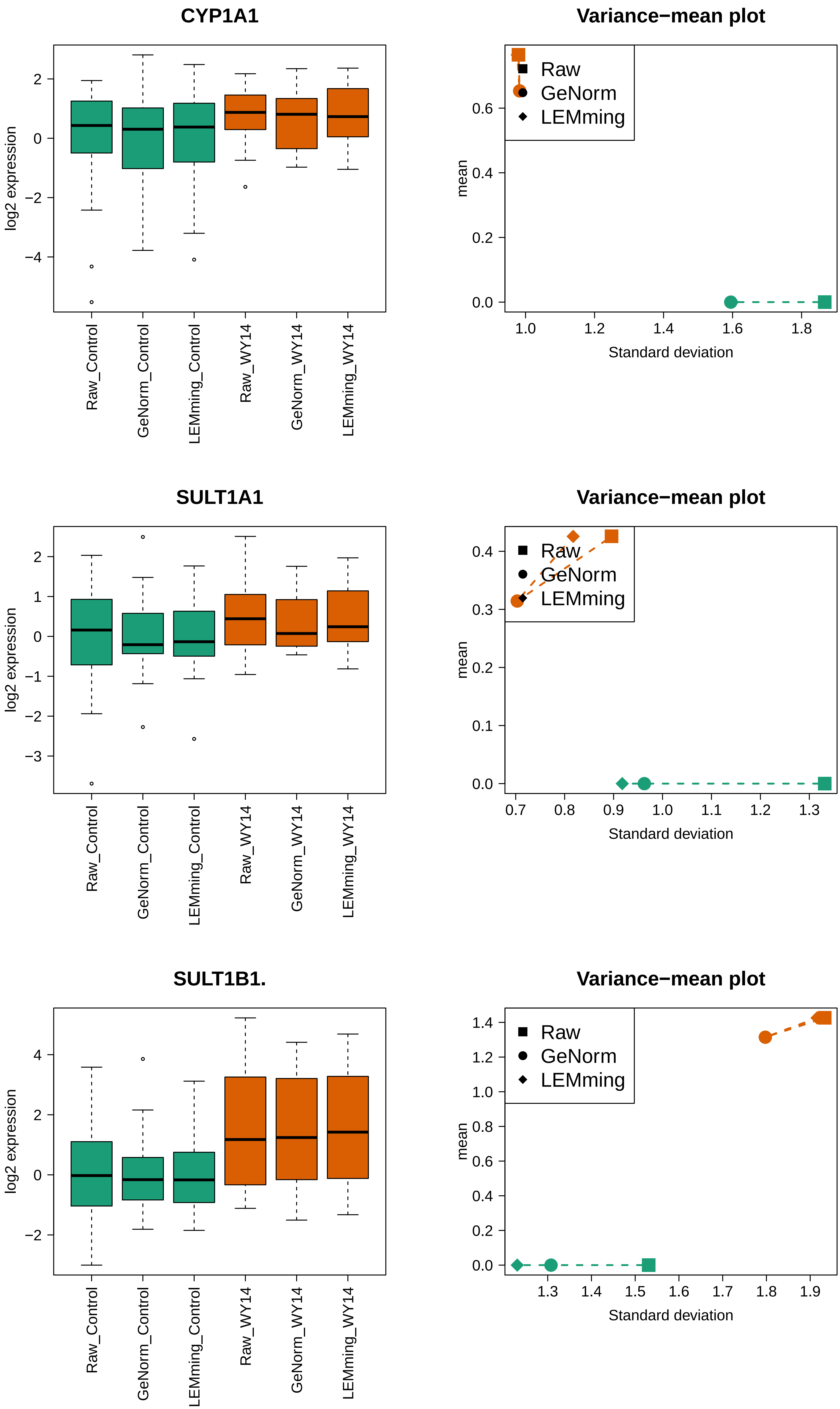
GeNorm_WY14
LEMming_WY14
Variance−mean plot
mean
Standard deviation
Raw
GeNorm
LEMming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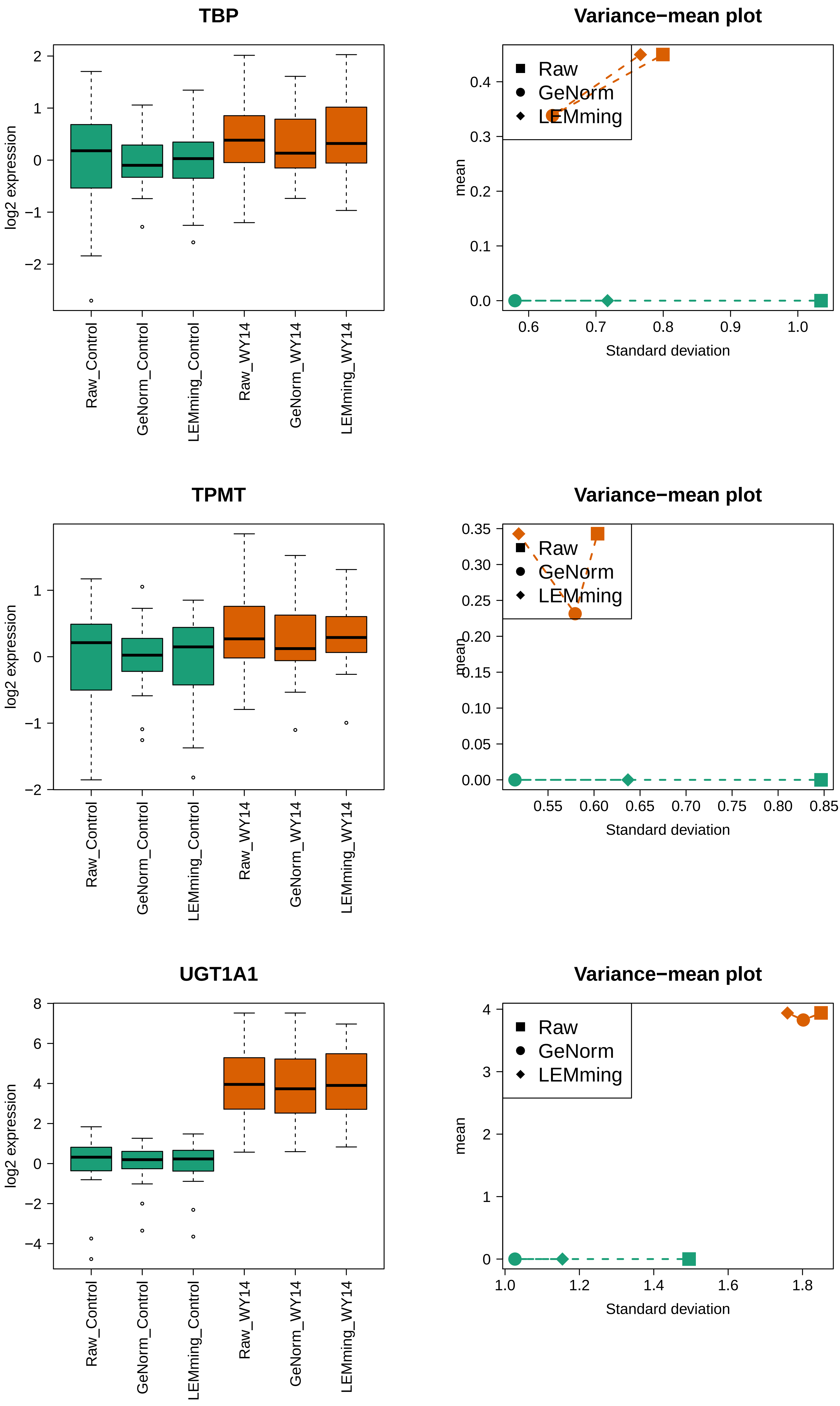
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
Standard deviation
SULT1A1
log2 expression
Raw_Control
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
Standard deviation
SULT1B1.
log2 expression
Raw_Control
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
Standard deviation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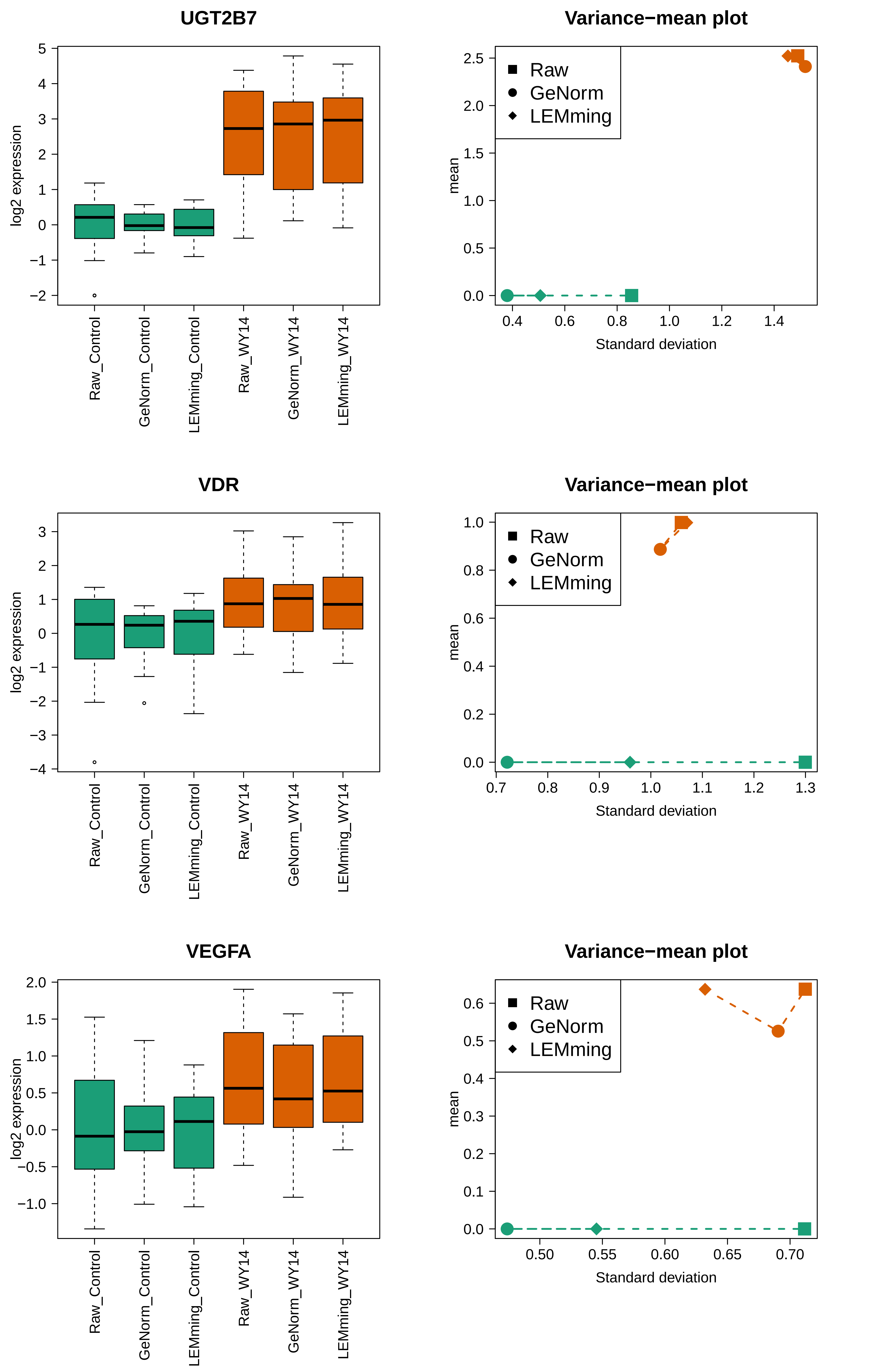
−1
−2
Raw_Control
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
0.4
0.3
0.2
0.1
0.0
0.6
0.7
0.8
0.9
1.0
Standard deviation
TPMT
log2 expression
1
0
−1
−2
Raw_Control
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.55
0.60
0.65
0.70
0.75
0.80
0.85
Standard deviation
UGT1A1
log2 expression
8
6
4
2
0
−2
−4
Raw_Control
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
4
3
2
1
0
1.0
1.2
1.4
1.6
1.8
Standard deviation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LEMming_Control
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GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
Standard deviation
VDR
log2 expression
Raw_Control
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
Standard deviation
VEGFA
log2 expression
Raw_Control
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LEMming_Control
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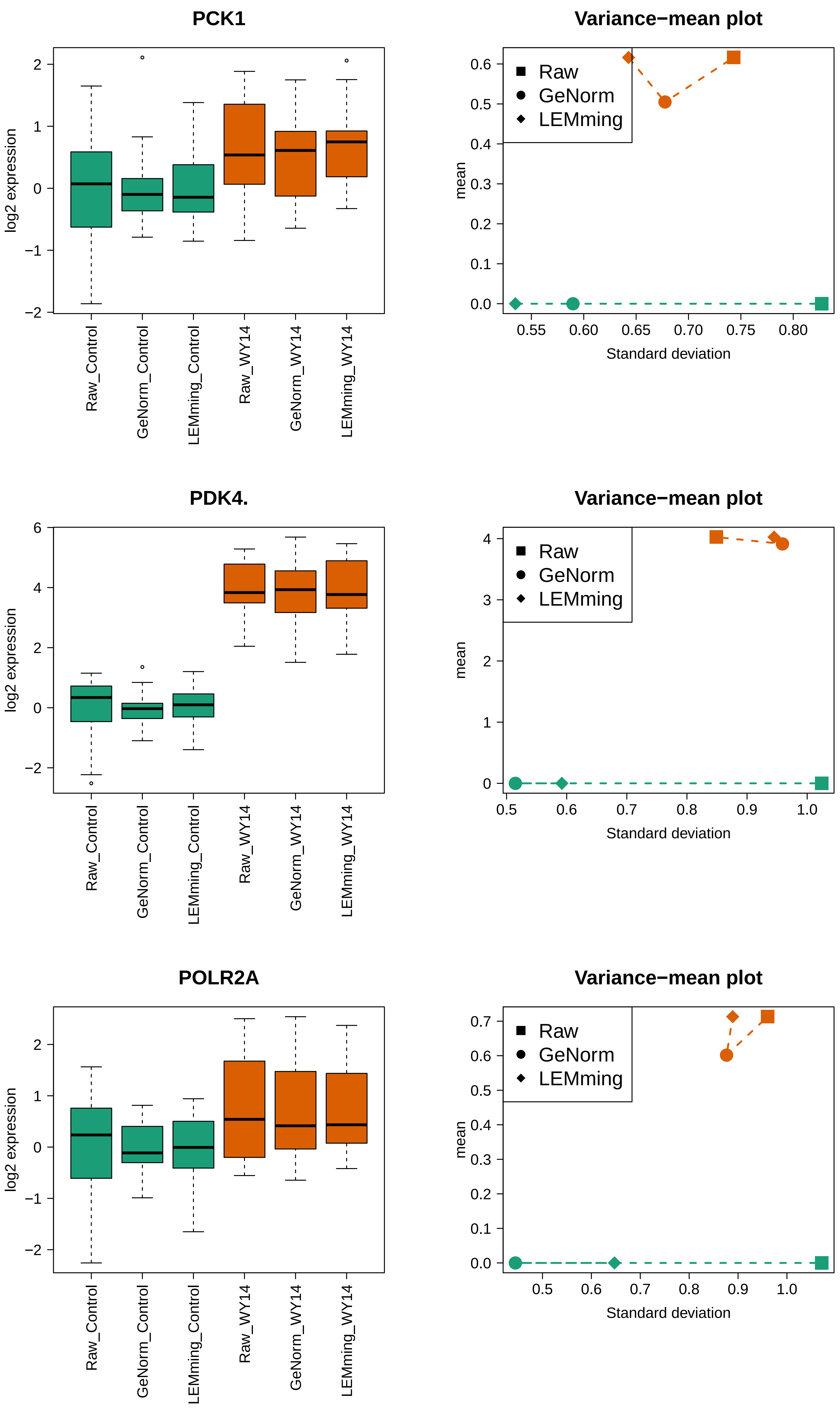
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
Standard deviation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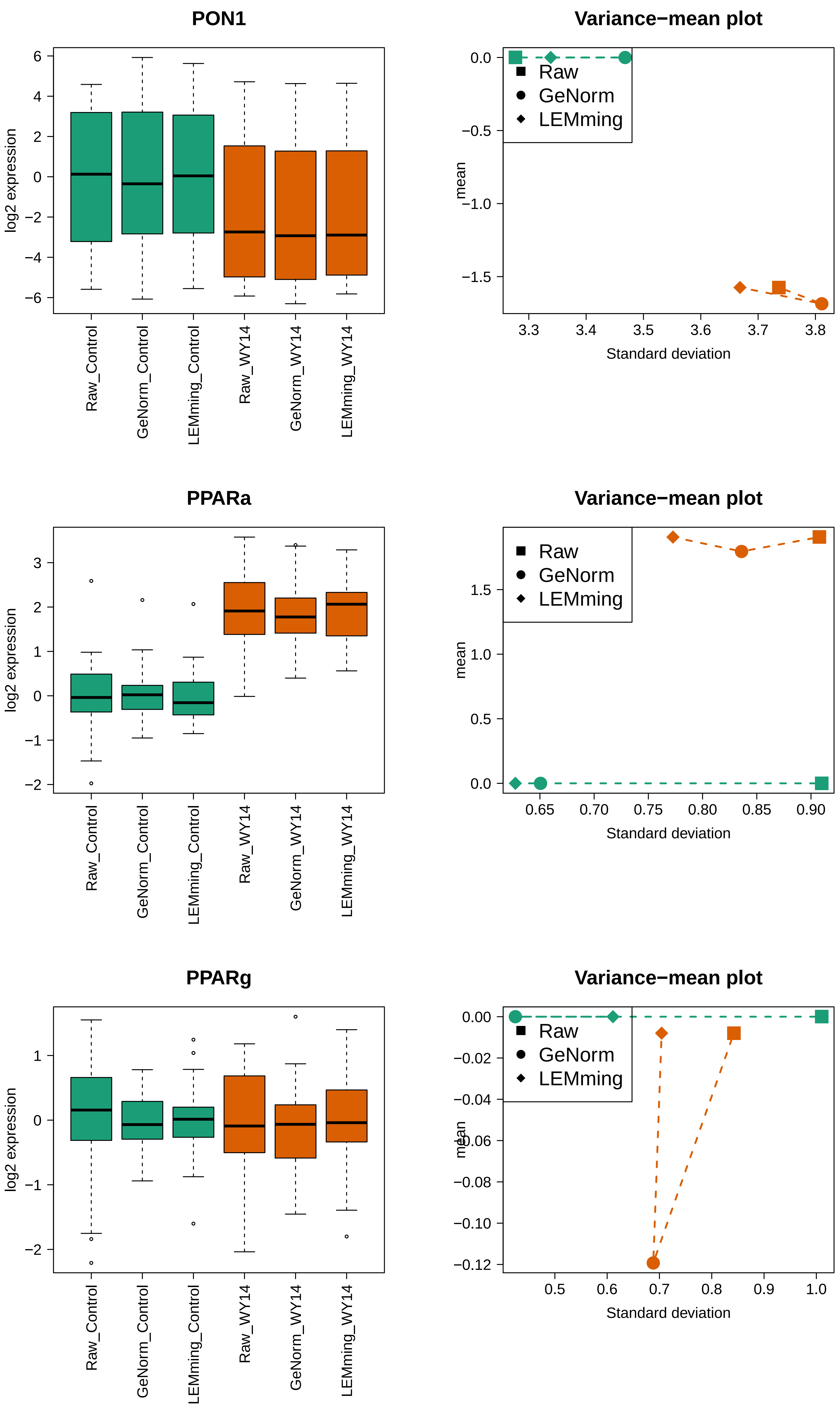
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
Standard deviation
PDK4.
log2 expression
Raw_Control
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
Standard deviation
POLR2A
log2 expression
Raw_Control
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
Standard deviation


PON1
log2 expression
6
4
2
0
−2
−4
−6
Raw_Control
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
mean
0.0
−0.5
−1.0
−1.5
Raw
GeNorm
LEMming
3.3
3.4
3.5
3.6
3.7
3.8
Standard deviation
PPARa
log2 expression
3
2
1
0
−1
−2
Raw_Control
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
mean
1.5
1.0
0.5
0.0
Raw
GeNorm
LEMming
0.65
0.70
0.75
0.80
0.85
0.90
Standard deviation
PPARg
log2 expression
1
0
−1
−2
Raw_Control
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
mean
0.00
−0.02
−0.04
−0.06
−0.08
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Raw
GeNorm
LEMming
0.5
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0.7
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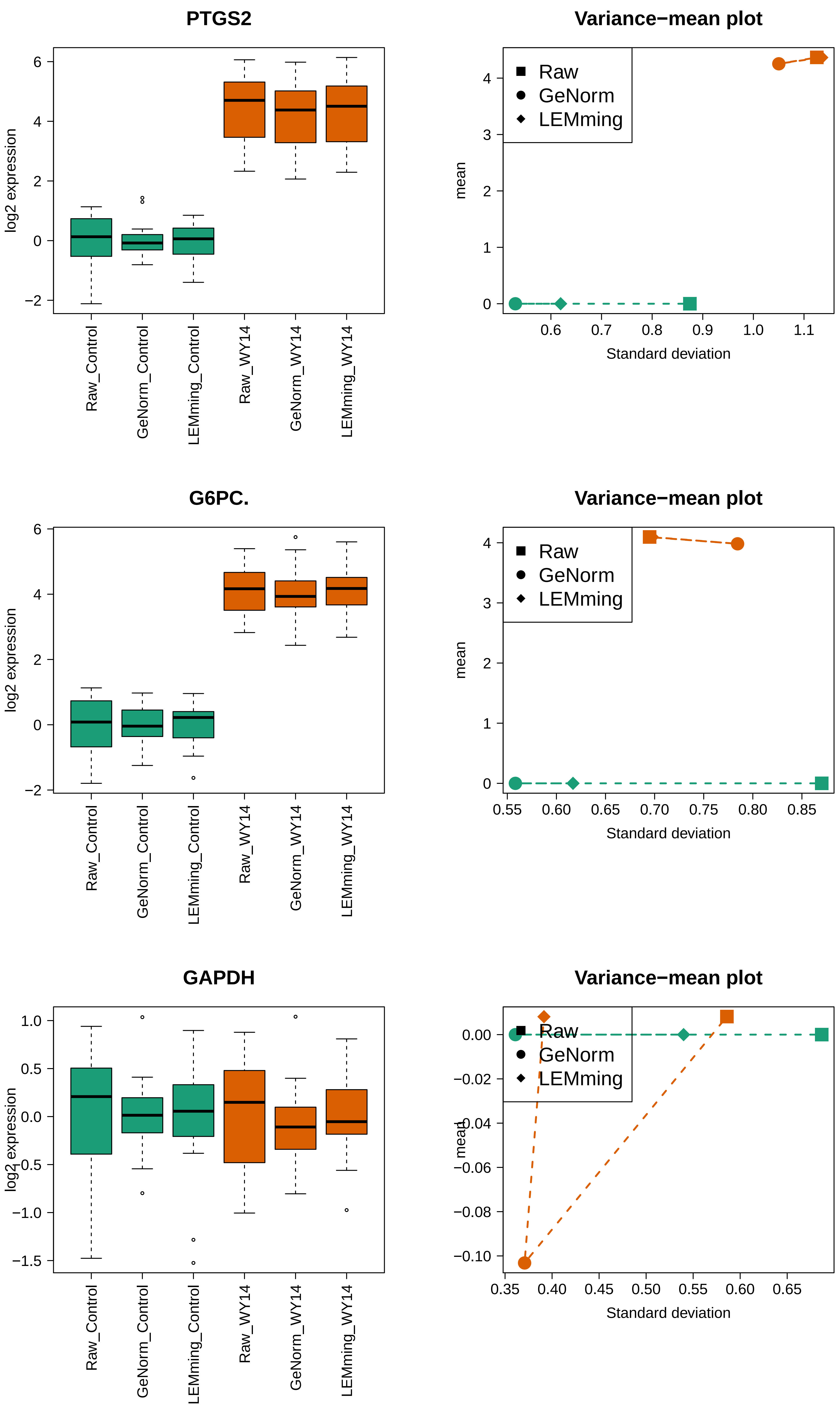
0.9
1.0
Standard deviation



PTGS2
Variance−mean plot
G6PC.
Variance−mean plot
GAPDH
Variance−mean plot
log2 expression
mean
Standard deviation
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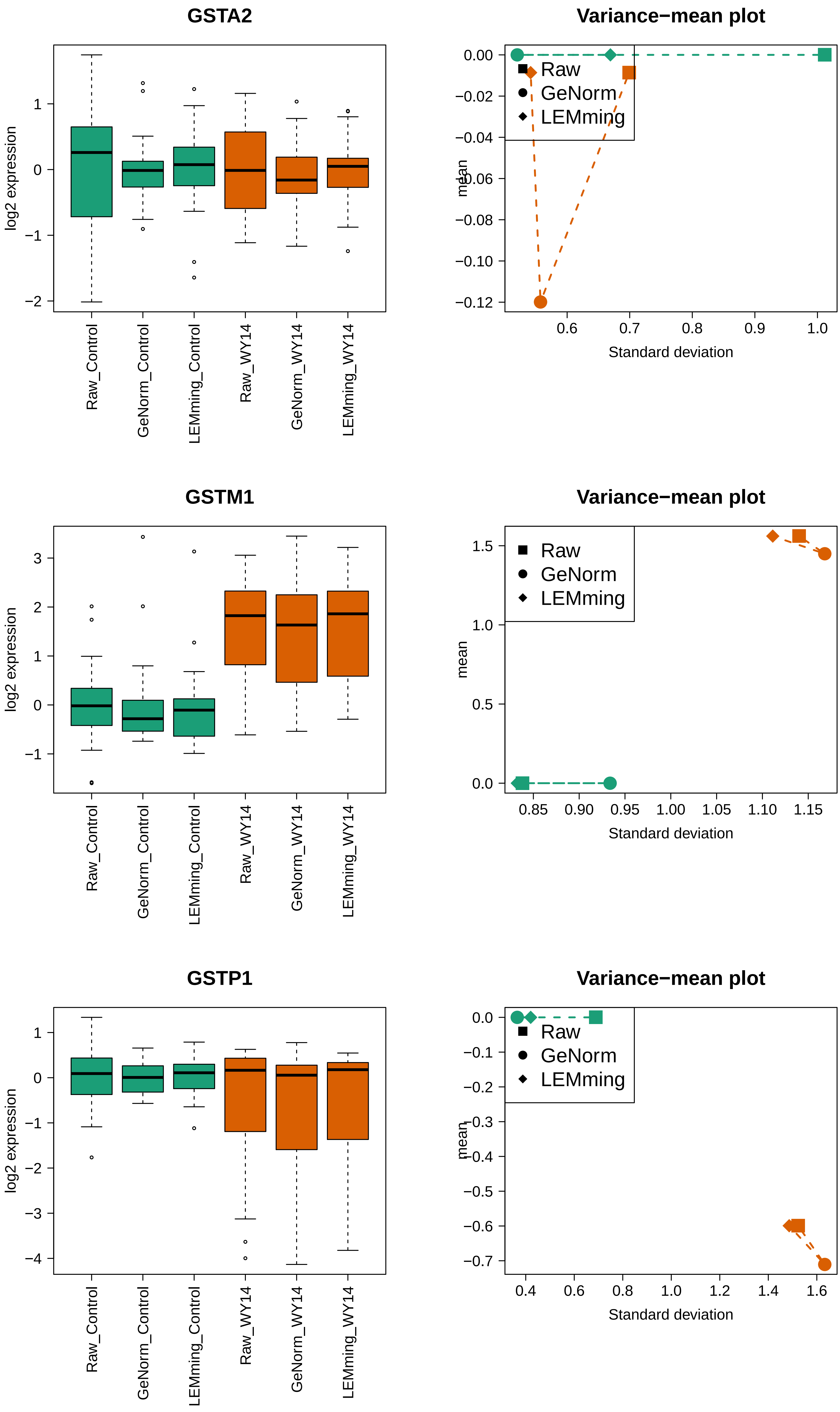
GeNorm
LEMming
Raw_Control
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14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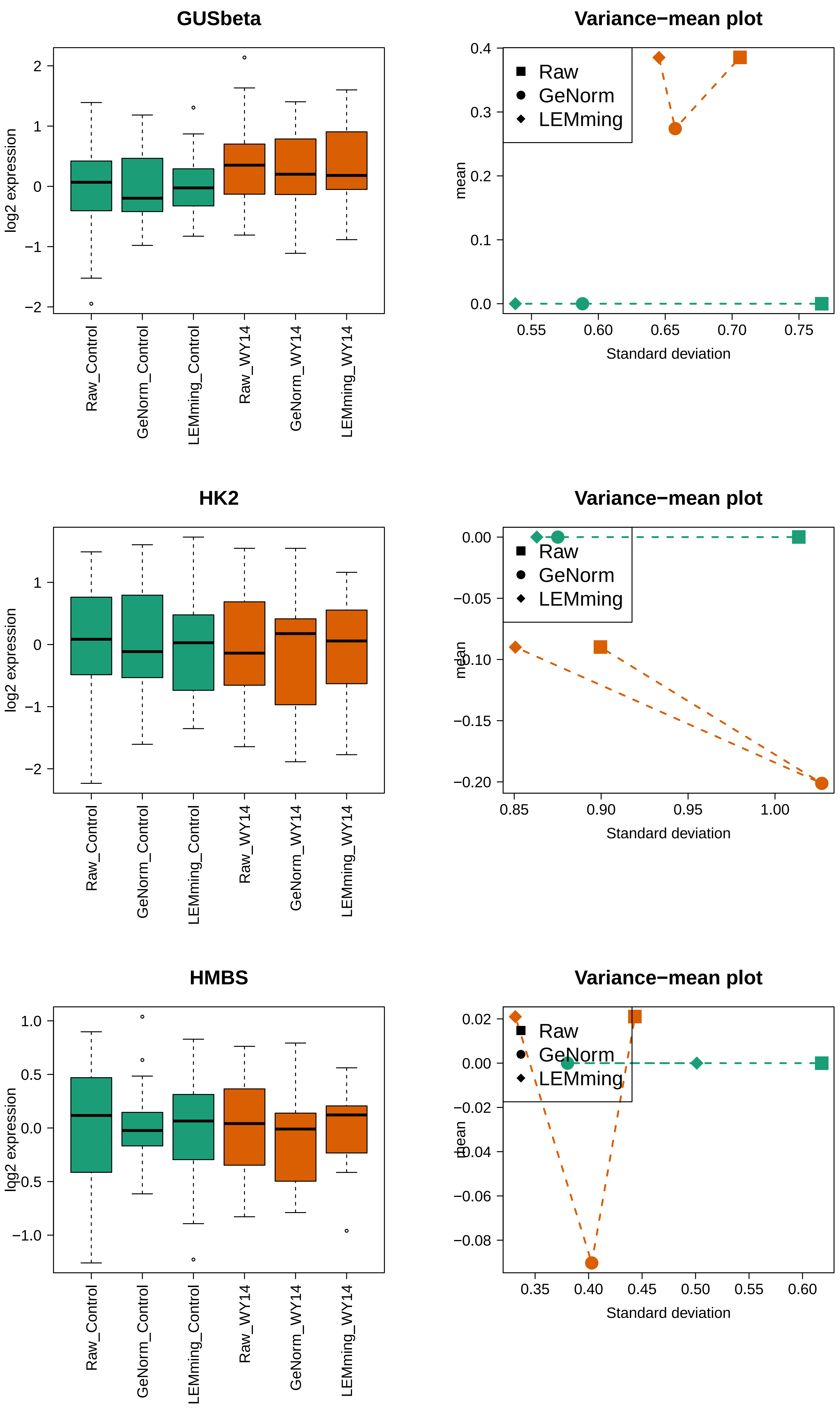
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
Standard deviation
GSTM1
log2 expression
Raw_Control
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
Standard deviation
GSTP1
log2 expression
Raw_Control
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
Standard deviation



GUSbeta
log2 expression
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GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
Standard deviation
HK2
log2 expression
Raw_Control
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
Standard deviation
HMBS
log2 expression
Raw_Control
GeNorm_Control
LEMming_Control
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GeNorm_WY14
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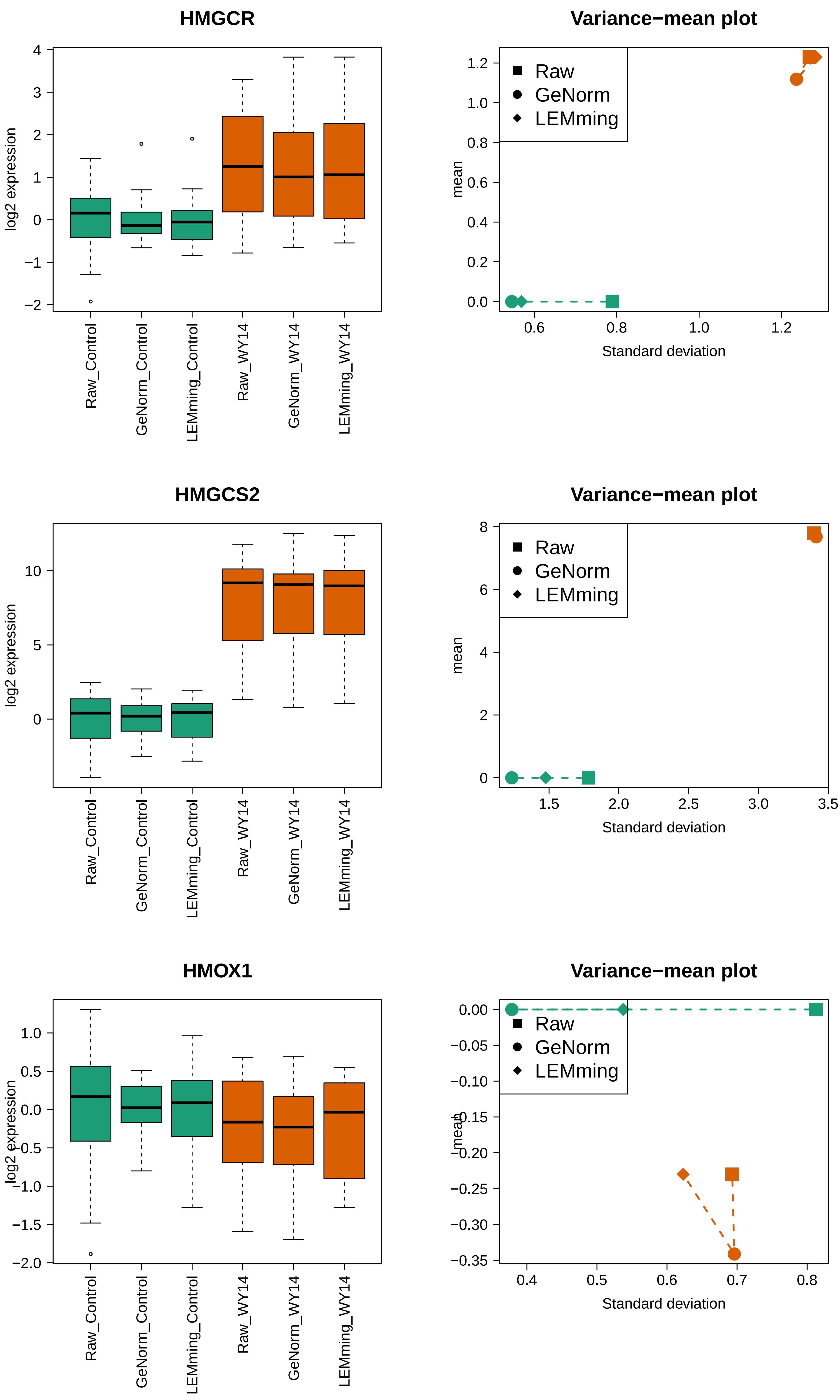
Variance−mean plot
Raw
GeNorm
LEMming
mean
Standard deviation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LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
Standard deviation
HMGCS2
log2 expression
Raw_Control
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
Standard deviation
HMOX1
log2 expression
Raw_Control
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Raw_WY14
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Variance−mean plot
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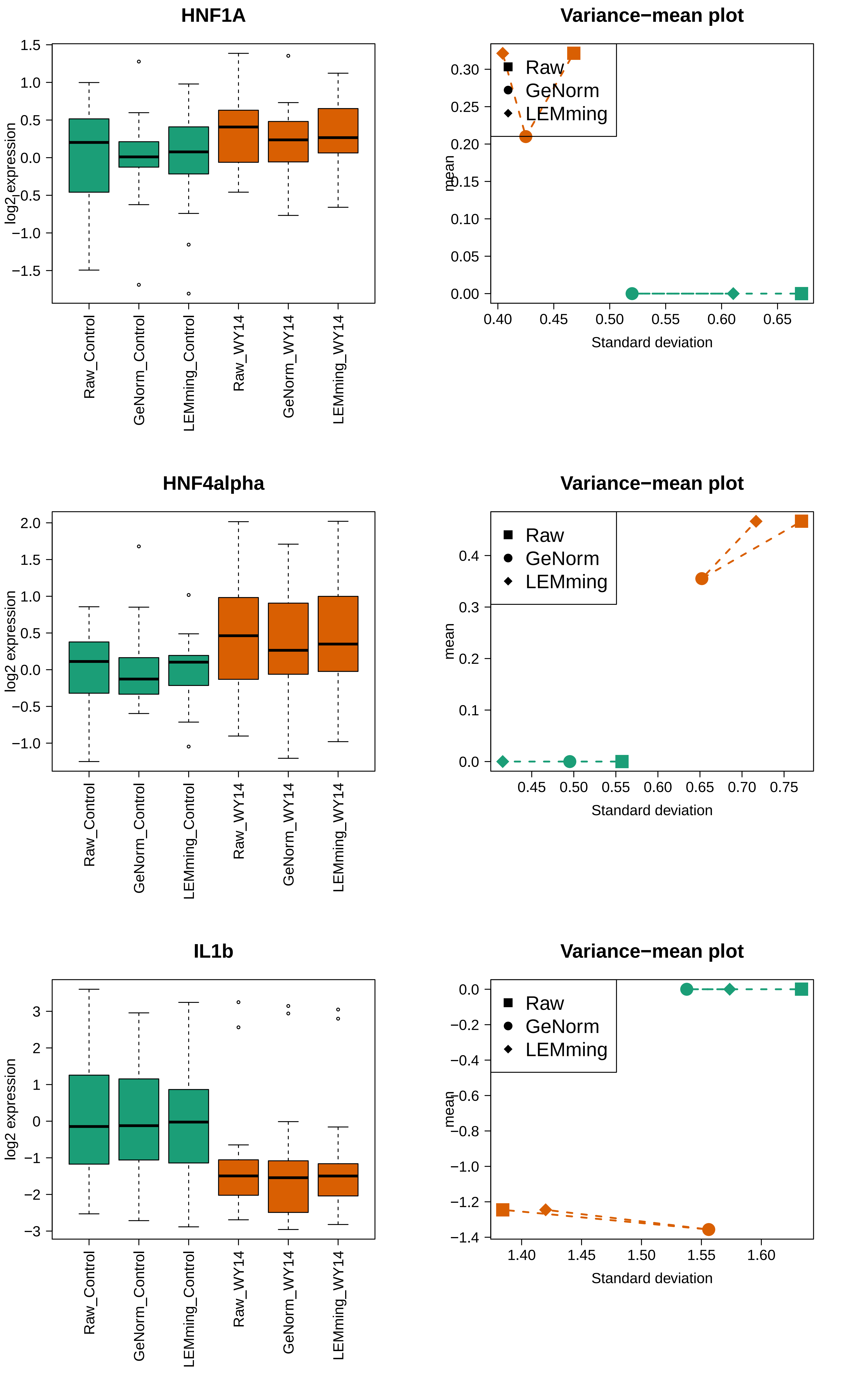
GeNorm
LEMming
mean
Standard deviation



HNF1A
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LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
Standard deviation
HNF4alpha
log2 expression
Raw_Control
GeNorm_Control
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14
Variance−mean plot
Raw
GeNorm
LEMming
mean
Standard deviation
IL1b
log2 expression
Raw_Control
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Variance−mean plot
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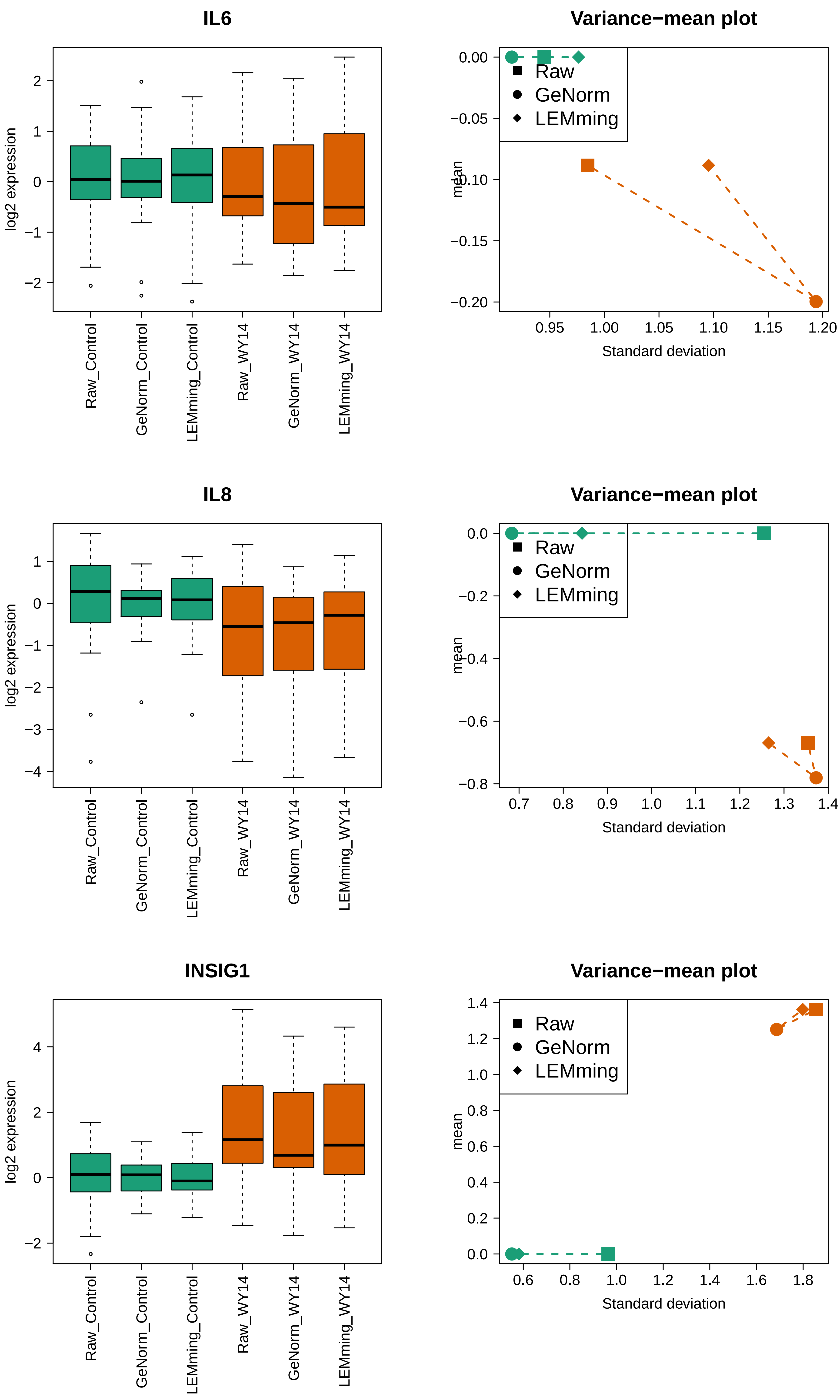
GeNorm
LEMming
mean
Standard deviation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GeNorm
LEMming
mean
Standard deviation
INSIG1
log2 expression
Raw_Control
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Variance−mean plot
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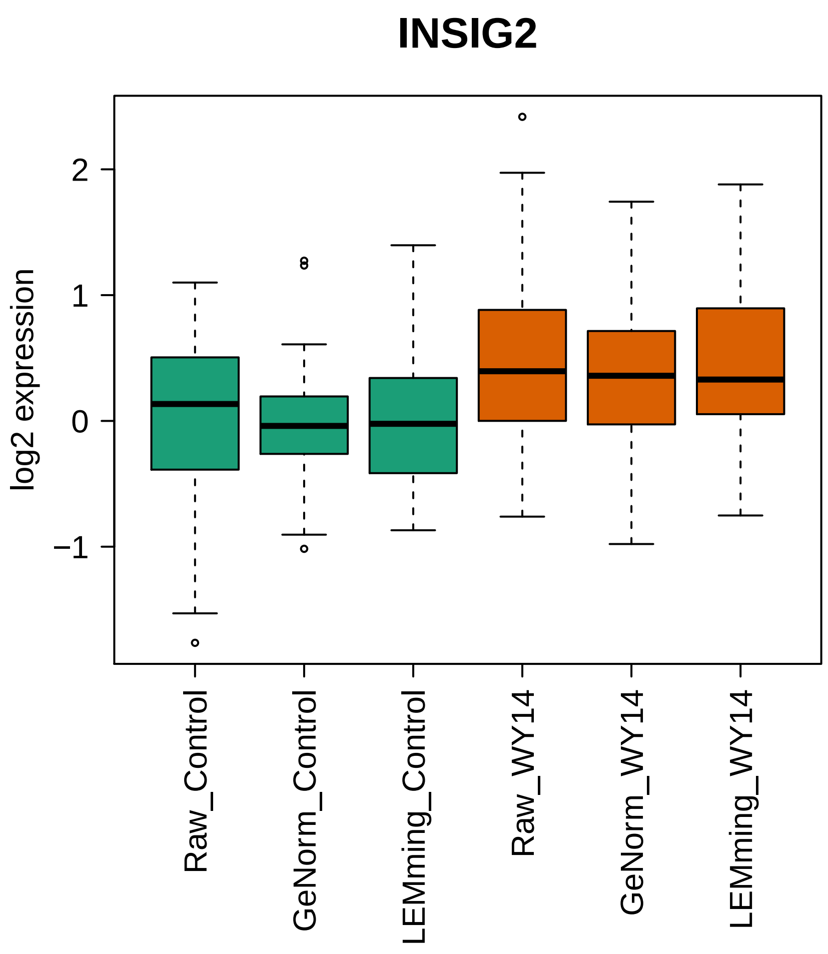
GeNorm
LEMming
mean
Standard deviation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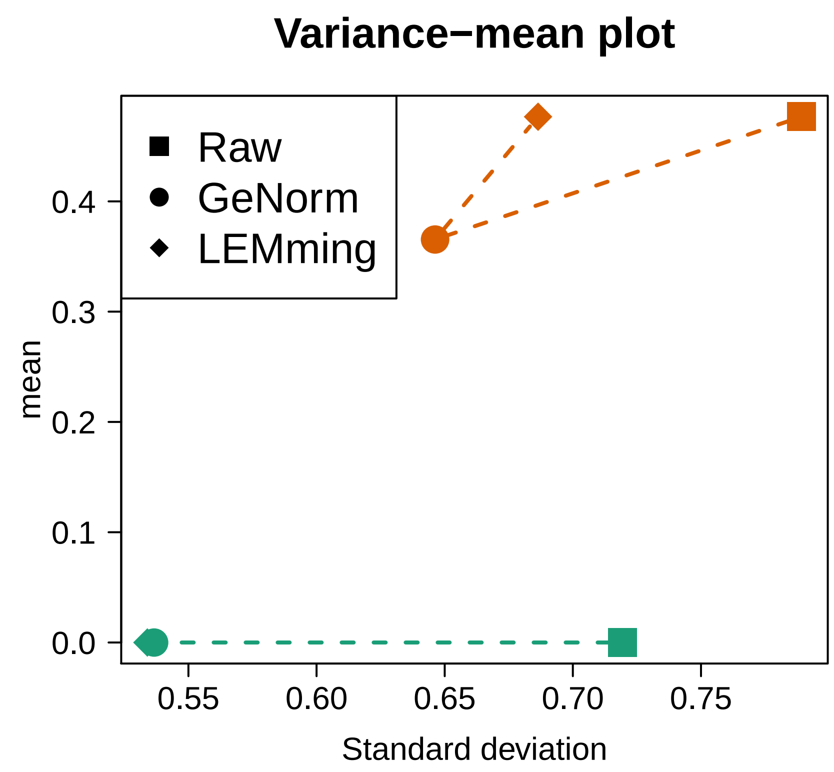
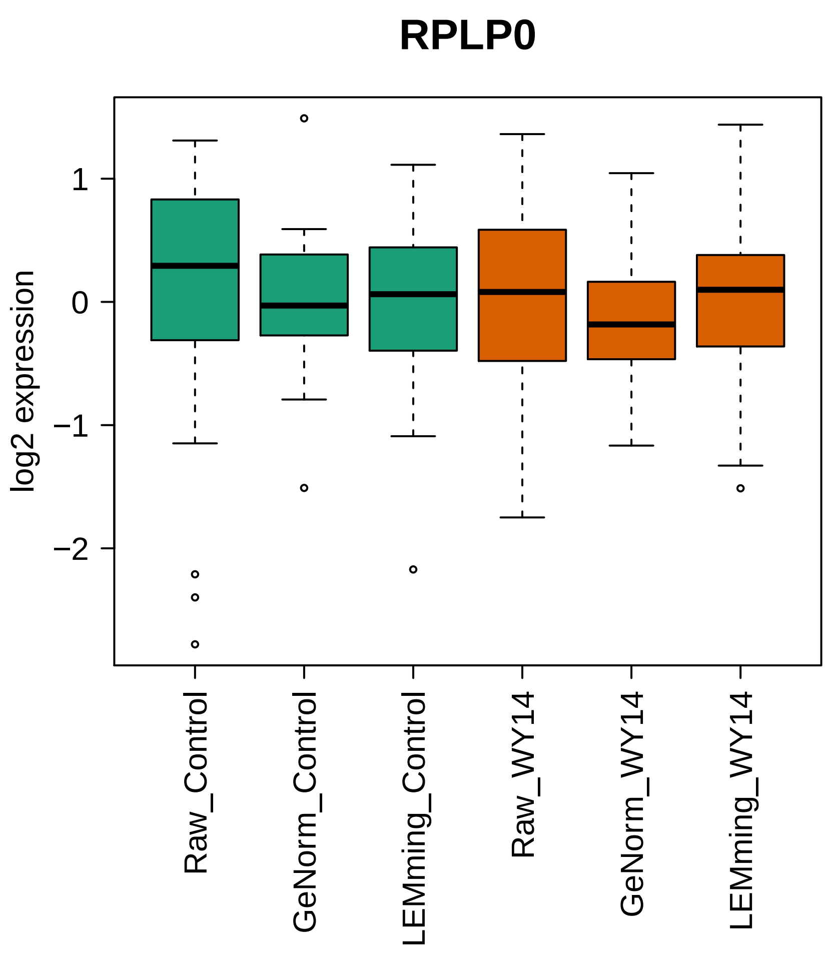
LEMming_Control
Raw_WY14
GeNorm_WY14
LEMming_WY14


Variance−mean plot
Raw
GeNorm
LEMming
mean
Standard deviation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LEMming_Control
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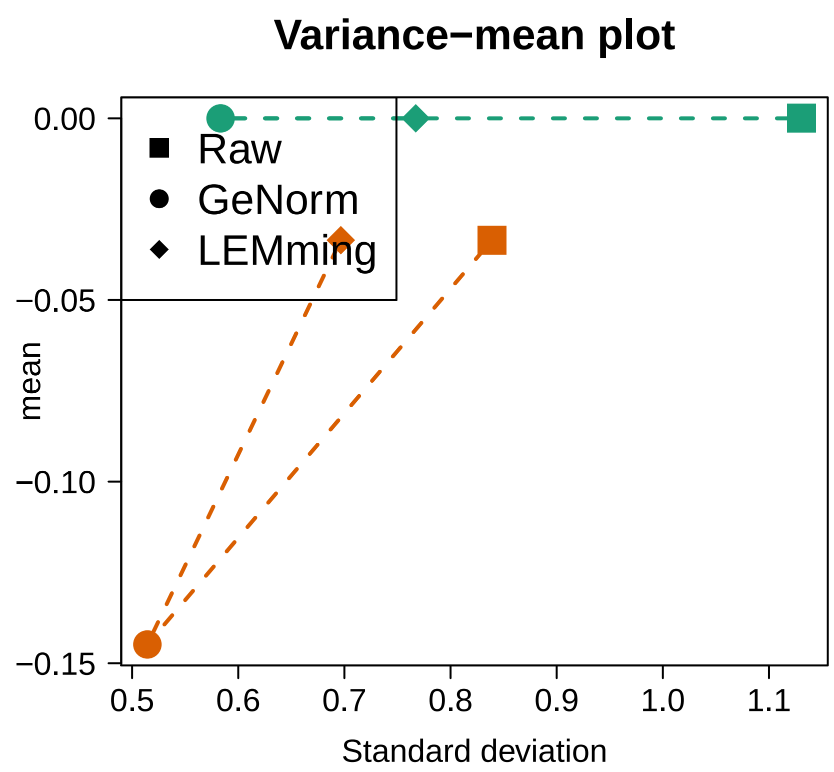
GeNorm_WY14
LEMming_WY14


Variance−mean plot
Raw
GeNorm
LEMming
mean
Standard deviation