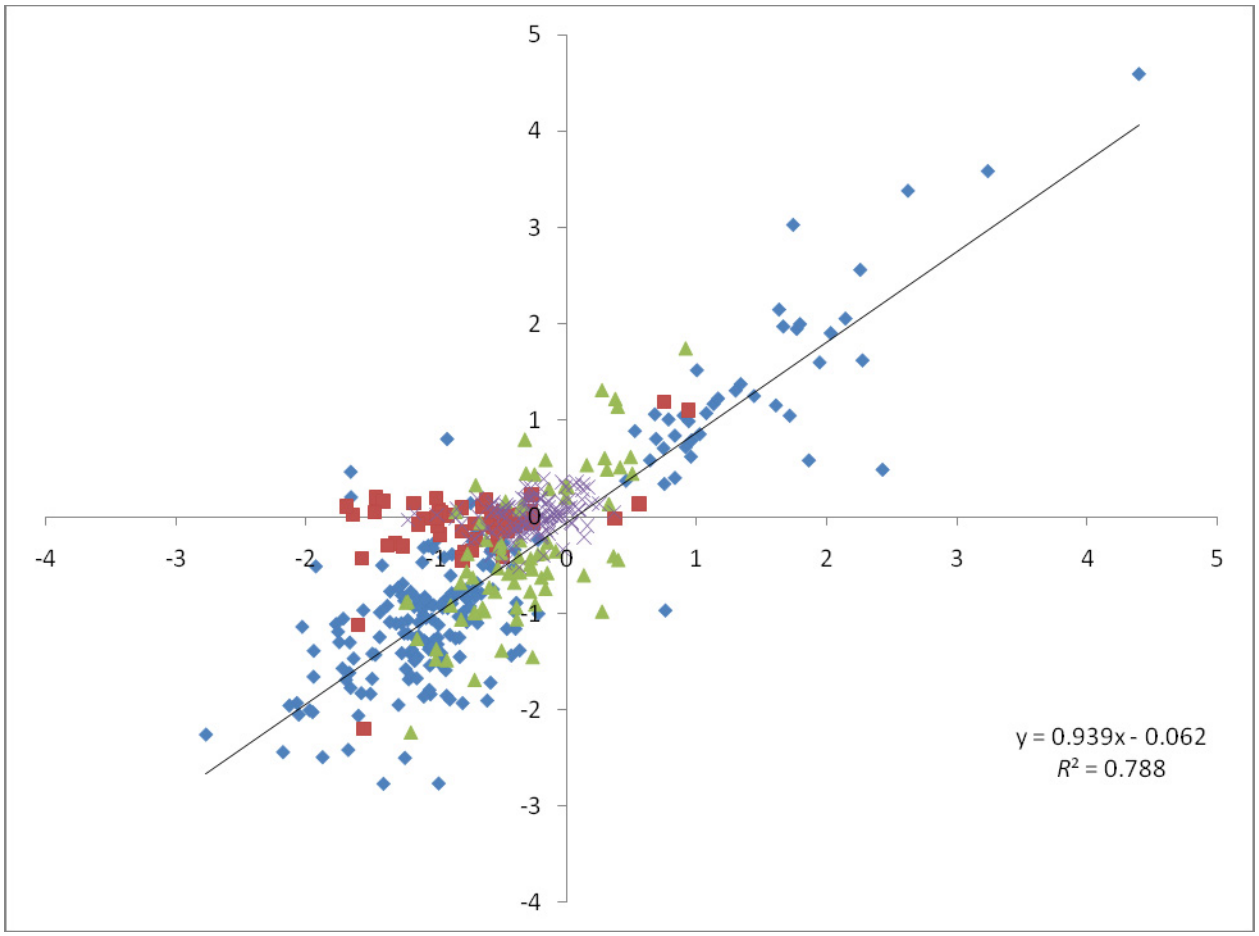
Replicates	Nano R^2	Affy R^2
M1:M2	0.9997	0.9969
M1:M3	0.9988	0.9969
M2:M3	0.9996	0.9972
PV1:PV2	0.9999	0.9855
PV1:PV3	0.9996	0.9978
PV2:PV3	0.9997	0.9860
Average	0.9995	0.9934
s.d.	0.0004	0.0059

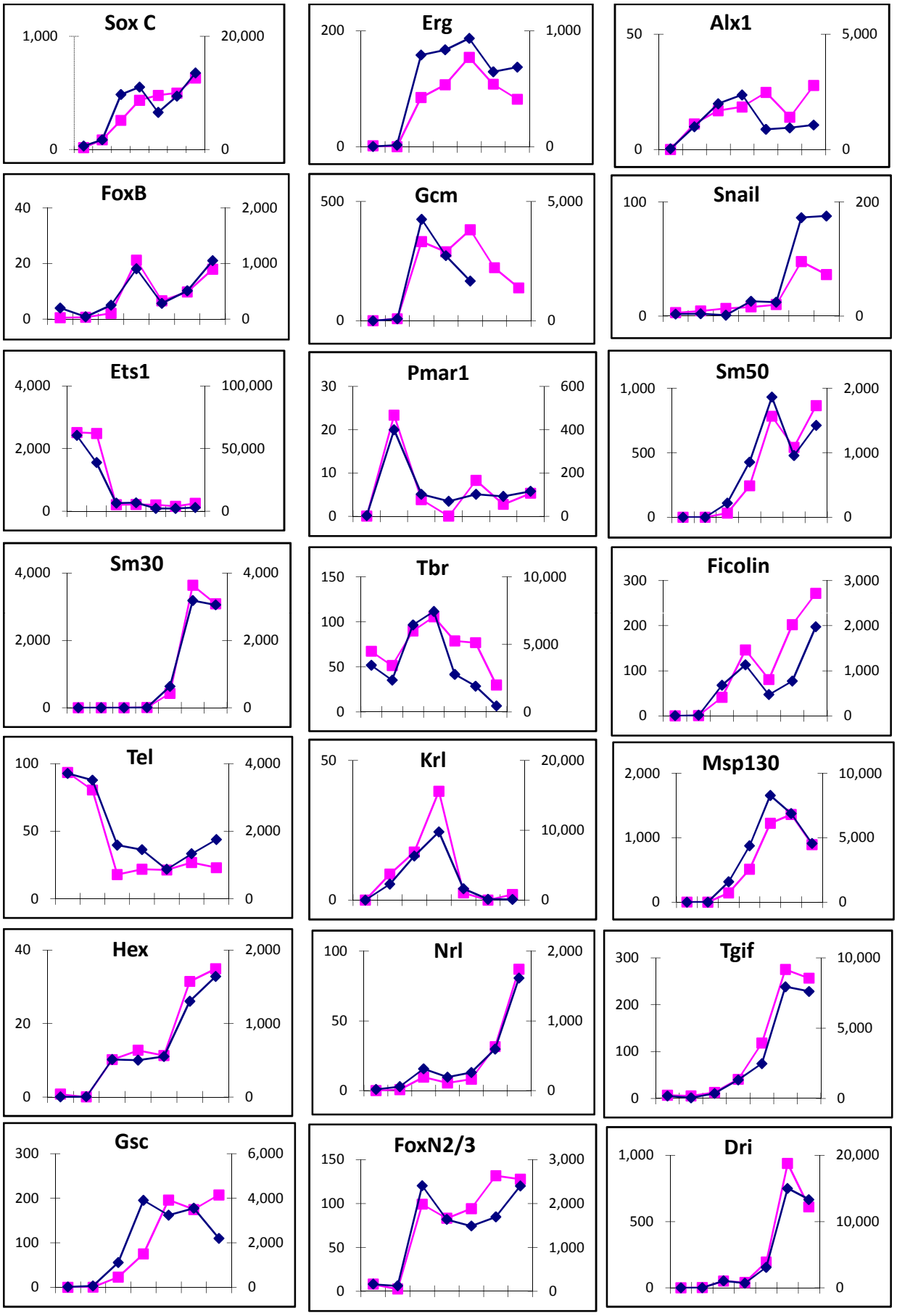
Table I. ***Correlation of technical replicates between NanoString and microarrays***

The correlation coefficients of linear regression to all possible pairwise comparisons of replicate analyses are shown. M1:M2 corresponds to a comparison of RNA from mock-infected cells between replicate assays 1 and 2. The results of both NanoString and Affymetrix assays are shown. The Affymetrix data was based on the most 3' probe sets that matched the REFSEQ accession numbers (see Methods). The average and standard deviations of the correlation coefficient (R^2) for both assays across all pair-wise comparisons are shown in the bottom two rows of the table.



Supplementary Figure 1: ***Comparison of fold change results for all 509 genes***

Scatter plot of log2 fold change for 317 genes that were measured by both NanoString and Affymetrix platforms. Genes are color-coded based on the significance of their fold change values (P≤0.05) in either both platforms (♦), NanoString platform only (■), Affymetrix platform only (▲), or neither platform (X). The *R*2 value shown represents the correlation of fold changes of genes that were found to be significant in both NanoString and microarray platforms.


Sox C
1,000
0
20,000
0
Erg
200
0
1,000
0
Alx1
50
0
5,000
0
FoxB
40
20
0
2,000
1,000
0
Gcm
500
0
5,000
0
Snail
100
0
200
0
Ets1
4,000
2,000
0
100,000
50,000
0
Pmar1
30
20
10
0
600
400
200
0
Sm50
1,000
500
0
2,000
1,000
0
Sm30
4,000
2,000
0
4,000
2,000
0
Tbr
150
100
50
0
10,000
5,000
0
Ficolin
300
200
100
0
3,000
2,000
1,000
0
Tel
100
50
0
4,000
2,000
0
Krl
50
0
20,000
10,000
0
Msp130
2,000
1,000
0
10,000
5,000
0
Hex
40
20
0
2,000
1,000
0
Nrl
100
50
0
2,000
1,000
0
Tgif
300
200
100
0
10,000
5,000
0
Gsc
300
200
100
0
6,000
4,000
2,000
0
FoxN2/3
150
100
50
0
3,000
2,000
1,000
0
Dri
1,000
500
0
20,000
10,000
0

Supplemenatry Figure 2. ***Correlation between nCounter and real-time PCR.***

Individual line plots for 21 genes across 7 time points are shown. The normalized counts obtained from the NanoString system are shown (■) on the left-hand y-axis scale. Quantitative real-time PCR results in copies/embryo are shown (♦) on the right-hand y-axis. The 7 time points (x-axis) were 0h (egg), 9.3h, 18h, 24h, 33h, 48h, and 70h. All data has been normalized to the expression levels of the polyubiquitin gene. Real-time PCR data is shown in copies/embryo and the NanoString data is shown in normalized counts. A quantitative comparison of the nCounter system and real-time PCR (not shown) reveals that estimates of transcript number for some genes are similar in the two systems, whereas others disagree. The discrepancies are likely to reflect differences in the two platforms. The nCounter system is based on solution-hybridization kinetics, directly measures mRNA transcripts, and uses a standard curve in each reaction to estimate transcript copy number. In contrast, real-time PCR involves a reverse transcription step followed by amplification of a portion of the cDNA with specific primers, and transcript copy number is calculated relative to polyubiquitin expression levels[6].