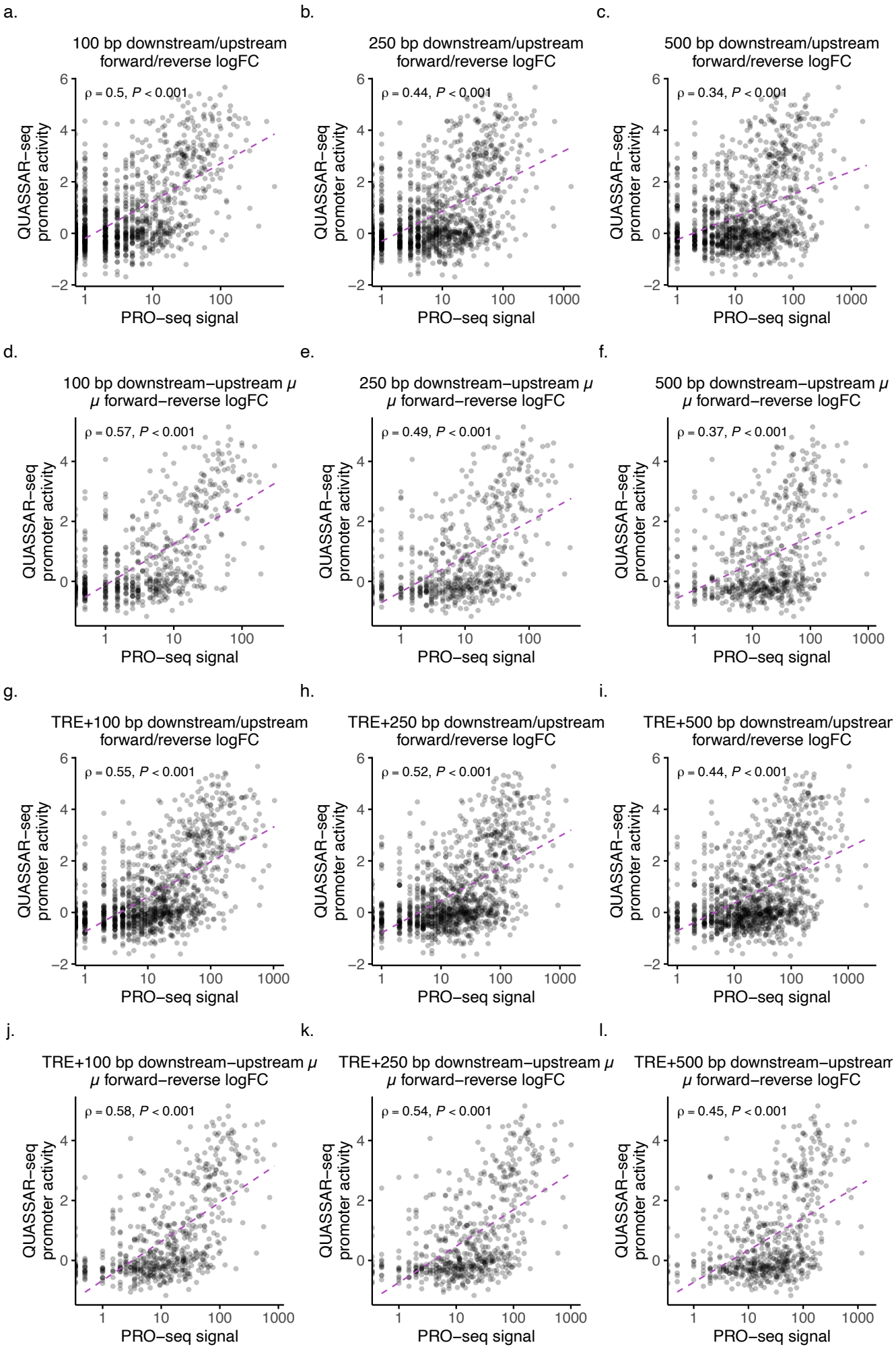
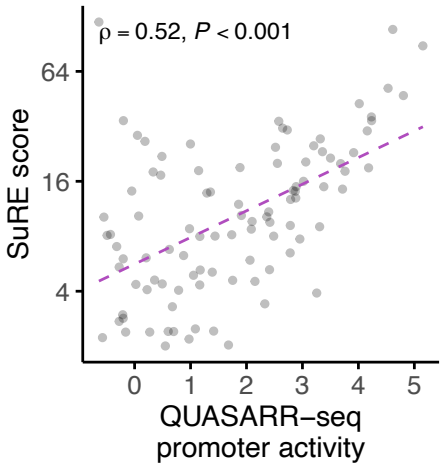
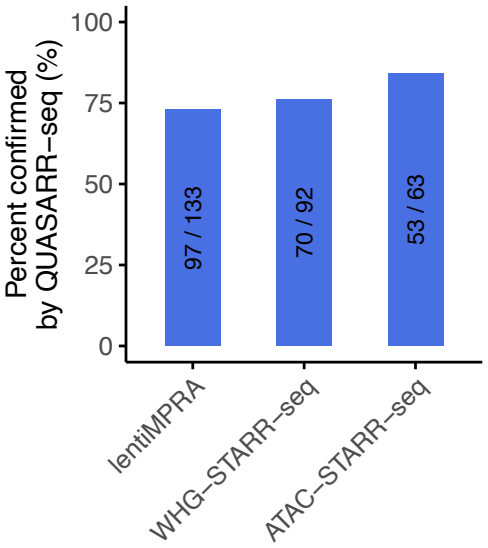

a.
100 bp downstream/upstream forward/reverse logFC
ρ = 0.5, P < 0.001
b.
250 bp downstream/upstream forward/reverse logFC
ρ = 0.44, P < 0.001
c.
500 bp downstream/upstream forward/reverse logFC
ρ = 0.34, P < 0.001
d.
100 bp downstream−upstream μ μ forward−reverse logFC
ρ = 0.57, P < 0.001
e.
250 bp downstream−upstream μ μ forward−reverse logFC
ρ = 0.49, P < 0.001
f.
500 bp downstream−upstream μ μ forward−reverse logFC
ρ = 0.37, P < 0.001
g.
TRE+100 bp downstream/upstream forward/reverse logFC
ρ = 0.55, P < 0.001
h.
TRE+250 bp downstream/upstream forward/reverse logFC
ρ = 0.52, P < 0.001
i.
TRE+500 bp downstream/upstrear forward/reverse logFC
ρ = 0.44, P < 0.001
j.
TRE+100 bp downstream−upstream μ μ forward−reverse logFC
ρ = 0.58, P < 0.001
k.
TRE+250 bp downstream−upstream μ μ forward−reverse logFC
ρ = 0.54, P < 0.001
l.
TRE+500 bp downstream−upstrean μ forward−reverse logFC
ρ = 0.45, P < 0.001
QUASSAR−seq promoter activity
PRO−seq signal

Supplementary Fig. 1: QUASARR-seq promoter activity comparisons with PRO-seq signal. **a-c**, Correlation between QUASARR-seq promoter activity, with elements in forward and reverse orientation treated as separate, and downstream (for forward) and upstream (for reverse) PRO-seq signal 100 (a), 250 (b), or 500 (c) bp from element. **d-f**, Correlation between QUASARR-seq promoter activity, using the mean of elements in forward and reverse orientation, and the mean of downstream and upstream PRO-seq signal 100 (d), 250 (e), or 500 (f) bp from element. **g-i**, Correlation between QUASARR-seq promoter activity, with elements in forward and reverse orientation treated as separate, and PRO-seq signal within the element and a 100 (g), 250 (h), or 500 (i) bp extension downstream (for forward) and upstream (for reverse) from element. **j-l**, Correlation between QUASARR-seq promoter activity, using the mean of elements in forward and reverse orientation, and PRO-seq signal within the element and the mean of downstream and upstream of 100 (j), 250 (k), or 500 (l) bp extensions from element. Spearman's rank correlation coefficient.

a.

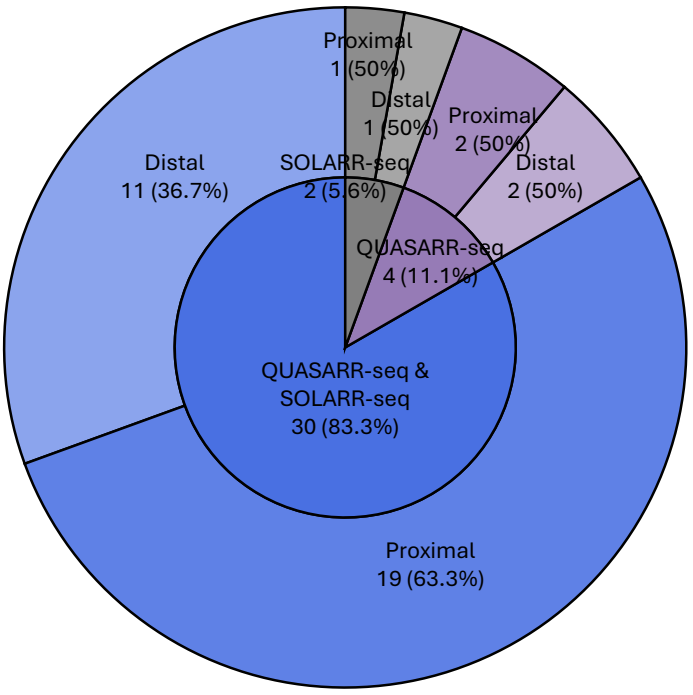

ρ = 0.52, P < 0.001
SuRE score
64
16
4
0
1
2
3
4
5
QUASARR–seq
promoter activity

b.

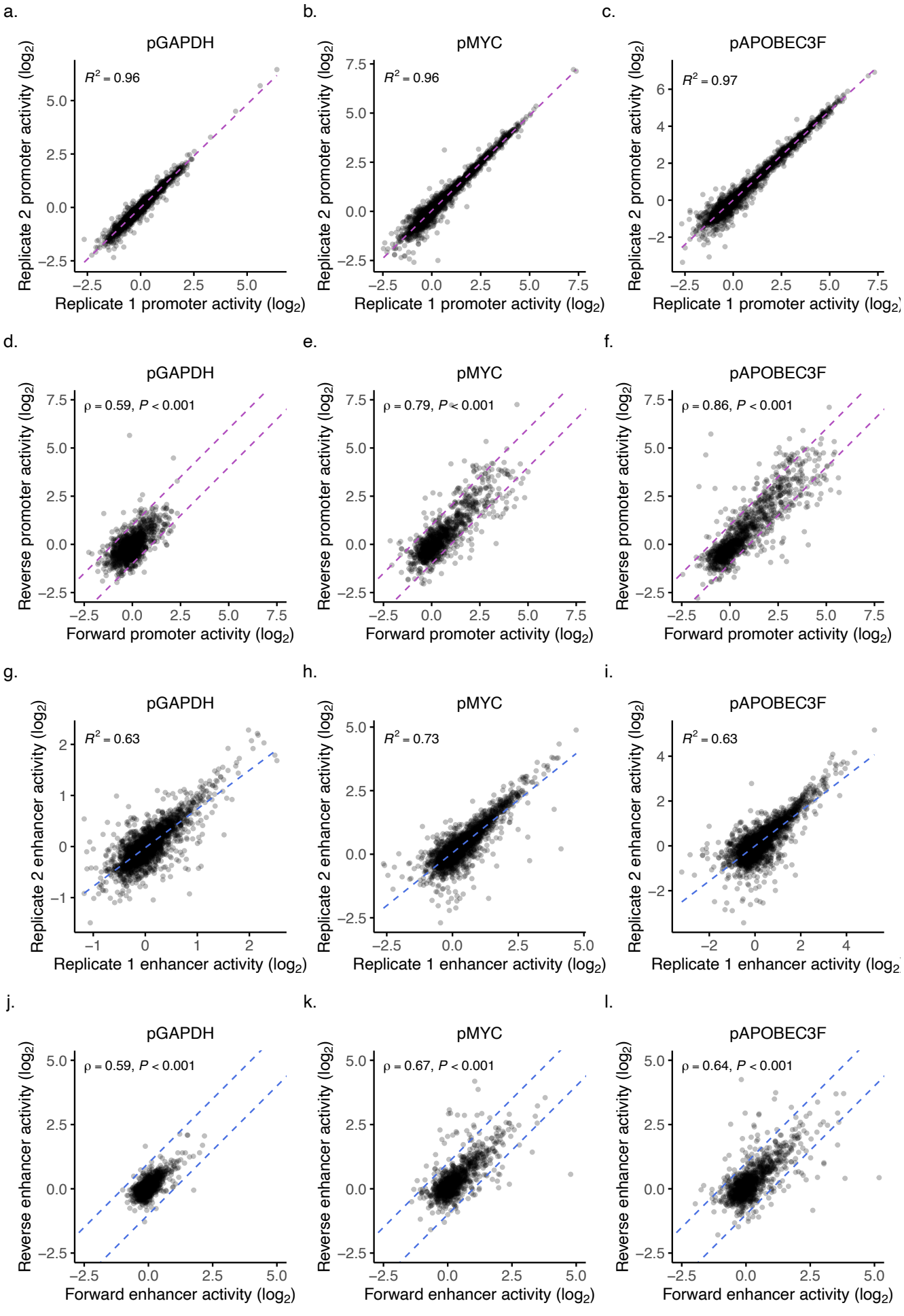

Percent confirmed
by QUASARR–seq (%)
100
75
50
25
0
97 / 133
70 / 92
53 / 63
lentiMPRA
WHG–STARR–seq
ATAC–STARR–seq

Supplementary Fig. 2: QUASARR-seq activity benchmarking with orthogonal assays.
a, Correlation between QUASARR-seq promoter activity and Survey of Regulatory Elements (SuRE) score (Spearman's $\rho = 0.52$, P-value < 0.001). **b**, Percent confirmed of lentiMPRA, WHG-STARR-seq, and ATAC-STARR-seq active enhancers by QUASARR-seq. Calculated based on 50% reciprocal overlap between elements across assays.

a.

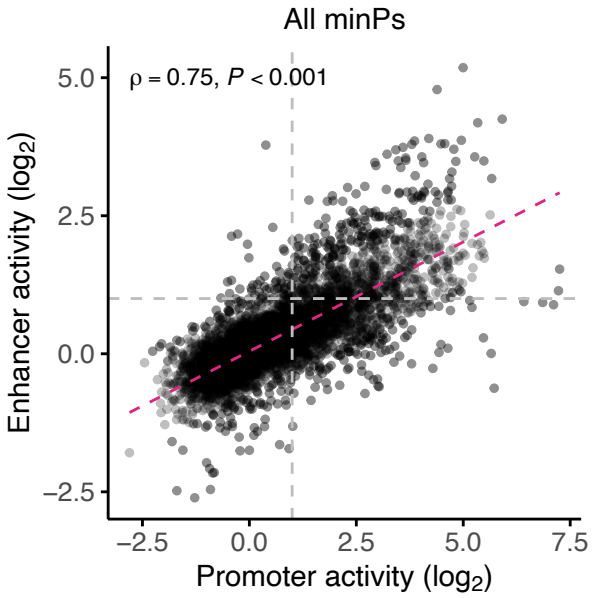

Proximal
1 (50%)
Distal
1 (50%)
Proximal
2 (50%)
Distal
2 (50%)
Distal
11 (36.7%)
SOLARR-seq
2 (5.6%)
QUASARR-seq
4 (11.1%)
QUASARR-seq &
SOLARR-seq
30 (83.3%)
Proximal
19 (63.3%)

Supplementary Fig. 3: Downstream sequence features do not establish element type.
a, Active enhancer calls by QUASARR-seq and SOLARR-seq parsed by GENCODE class.

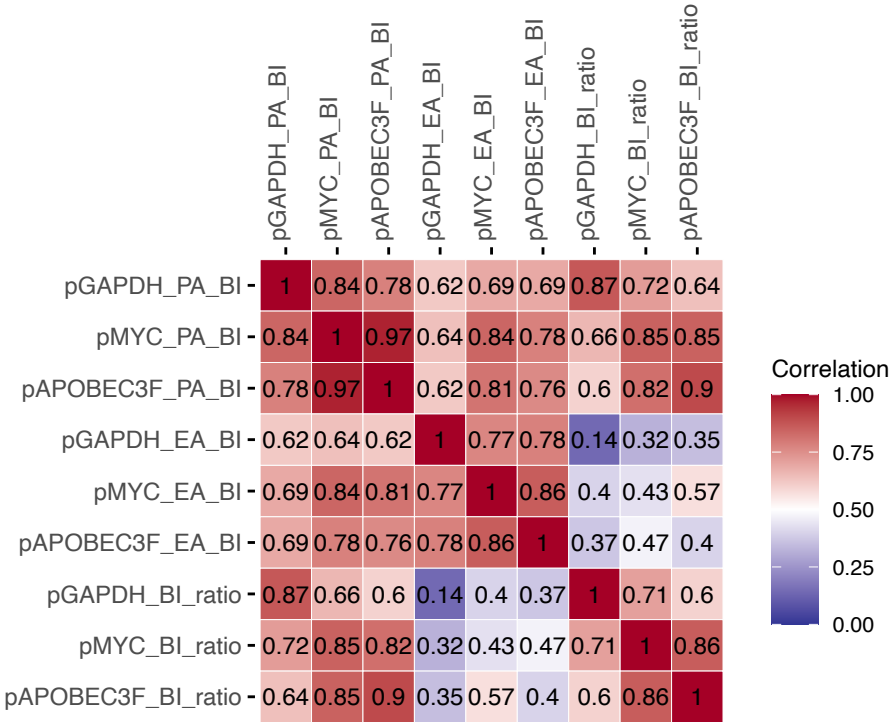

a.
pGAPDH
R^2 = 0.96
Replicate 2 promoter activity (log2)
Replicate 1 promoter activity (log2)
b.
pMYC
R^2 = 0.96
Replicate 2 promoter activity (log2)
Replicate 1 promoter activity (log2)
c.
pAPOBEC3F
R^2 = 0.97
Replicate 2 promoter activity (log2)
Replicate 1 promoter activity (log2)
d.
pGAPDH
ρ = 0.59, P < 0.001
Reverse promoter activity (log2)
Forward promoter activity (log2)
e.
pMYC
ρ = 0.79, P < 0.001
Reverse promoter activity (log2)
Forward promoter activity (log2)
f.
pAPOBEC3F
ρ = 0.86, P < 0.001
Reverse promoter activity (log2)
Forward promoter activity (log2)
g.
pGAPDH
R^2 = 0.63
Replicate 2 enhancer activity (log2)
Replicate 1 enhancer activity (log2)
h.
pMYC
R^2 = 0.73
Replicate 2 enhancer activity (log2)
Replicate 1 enhancer activity (log2)
i.
pAPOBEC3F
R^2 = 0.63
Replicate 2 enhancer activity (log2)
Replicate 1 enhancer activity (log2
j.
pGAPDH
ρ = 0.59, P < 0.001
Reverse enhancer activity (log2)
Forward enhancer activity (log2)
k.
pMYC
ρ = 0.67, P < 0.001
Reverse enhancer activity (log2)
Forward enhancer activity (log2)
l.
pAPOBEC3F
ρ = 0.64, P < 0.001
Reverse enhancer activity (log2)
Forward enhancer activity (log2)

Supplementary Fig. 4: minP QUASARR-seq data quality metrics.
a-c, Correlation of QUASARR-seq promoter activity measurements between replicates when paired with pGAPDH (a), pMYC (b), and pAPOBEC3F (c). **d-f**, Correlation of QUASARR-seq promoter activity measurements between elements cloned in forward and reverse orientations when paired with pGAPDH (d), pMYC (e), and pAPOBEC3F (f). **g-i**, Correlation of QUASARR-seq enhancer activity measurements between replicates when paired with pGAPDH (g), pMYC (h), and pAPOBEC3F (i). **j-l**, Correlation of QUASARR-seq enhancer activity measurements between elements cloned in forward and reverse orientations when paired with pGAPDH (j), pMYC (k), and pAPOBEC3F (l).

a.


All minPs
ρ = 0.75, P < 0.001
Enhancer activity (log2)
Promoter activity (log2)
5.0
2.5
0.0
−2.5
−2.5
0.0
2.5
5.0
7.5

b.


pGAPDH_PA_BI
pMYC_PA_BI
pAPOBEC3F_PA_BI
pGAPDH_EA_BI
pMYC_EA_BI
pAPOBEC3F_EA_BI
pGAPDH_BI_ratio
pMYC_BI_ratio
pAPOBEC3F_BI_ratio
1 0.84 0.78 0.62 0.69 0.69 0.87 0.72 0.64
0.84 1 0.97 0.64 0.84 0.78 0.66 0.85 0.85
0.78 0.97 1 0.62 0.81 0.76 0.6 0.82 0.9
0.62 0.64 0.62 1 0.77 0.78 0.14 0.32 0.35
0.69 0.84 0.81 0.77 1 0.86 0.4 0.43 0.57
0.69 0.78 0.76 0.78 0.86 1 0.37 0.47 0.4
0.87 0.66 0.6 0.14 0.4 0.37 1 0.71 0.6
0.72 0.85 0.82 0.32 0.43 0.47 0.71 1 0.86
0.64 0.85 0.9 0.35 0.57 0.4 0.6 0.86 1
Correlation
1.00
0.75
0.50
0.25
0.00

Supplementary Fig. 5: Comparisons of the different minP QUASARR-seq datasets.
a, Correlation between element promoter and enhancer activities when paired with all minPs (Spearman's $\rho = 0.75$, P-value < 0.001). **b**, Heatmap showing correlation between promoter activity (PA), enhancer activity (EA), and boost index (BI) ratio of elements when paired with the minPs pGAPDH, pMYC, and pAPOBEC3F.