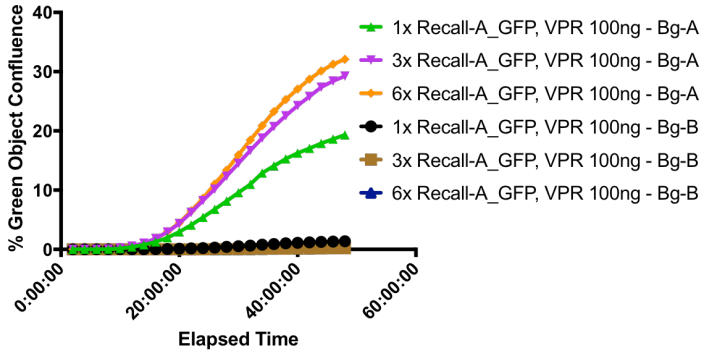
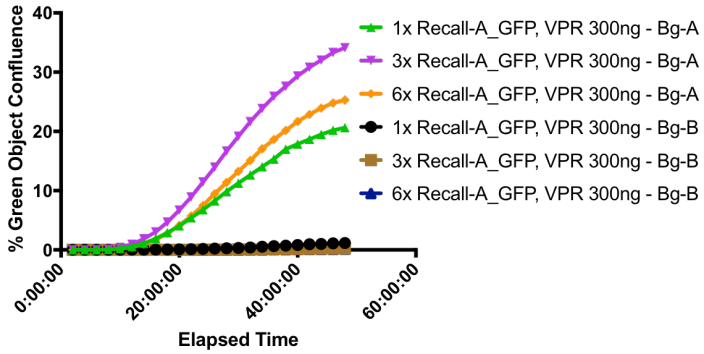
Supplementary Figure S1. Bg-A landing pad array assembly. The 3x barcode landing pad arrays were assembled by first annealing complimentary oligonucleotides containing the barcodes of interest and PAM site along with the specified overhangs A-F (a). When combined, these specified overhangs drive assembly of the individual double stranded barcodes to both make the 3x barcode array as well as direct integration into the BbsI digested Recall plasmid (b). A similar schemes was used to assemble the 6x barcode array.

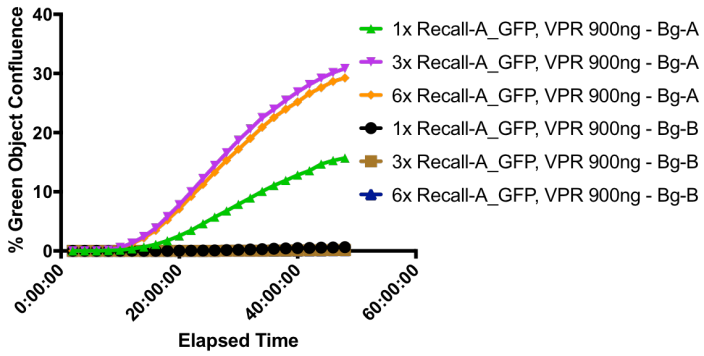
Barcode A 1/3/6x Array with 100ng dCas9-VPR



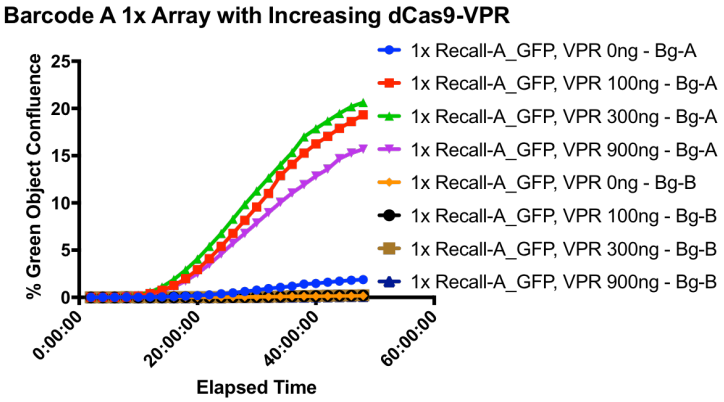
Barcode A 1/3/6x Array with 300ng dCas9-VPR

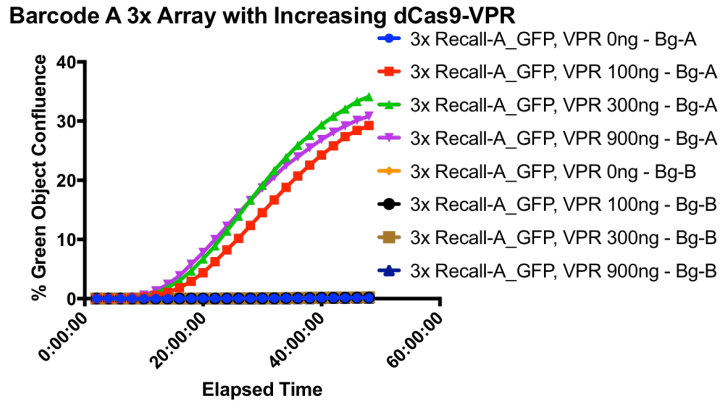


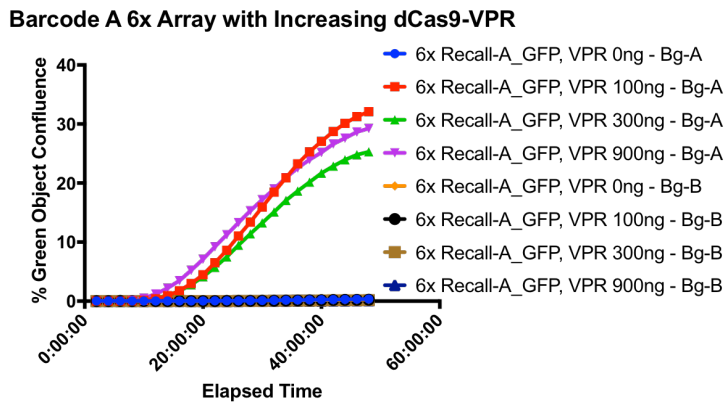
Barcode A 1/3/6x Array with 900ng dCas9-VPR



Supplementary Figure S2. Lineage specific gene activation efficiency of 1x, 3x, 6x barcode landing pads at different concentrations of dCas9-VPR. Time lapse fluorescent analysis of percent green object confluence of HEK293Ts Bg-A and Bg-B populations co-transfected with dCas9-VPR and 80ng of Recall-A_GFP plasmids with a 1x, 3x, or 6x barcode array in a 24 well plate. These graphs compare recall activation efficiency between Recall-A_GFP plasmids with a 1x, 3x, or 6x barcode array at given dCas9-VPR amounts.



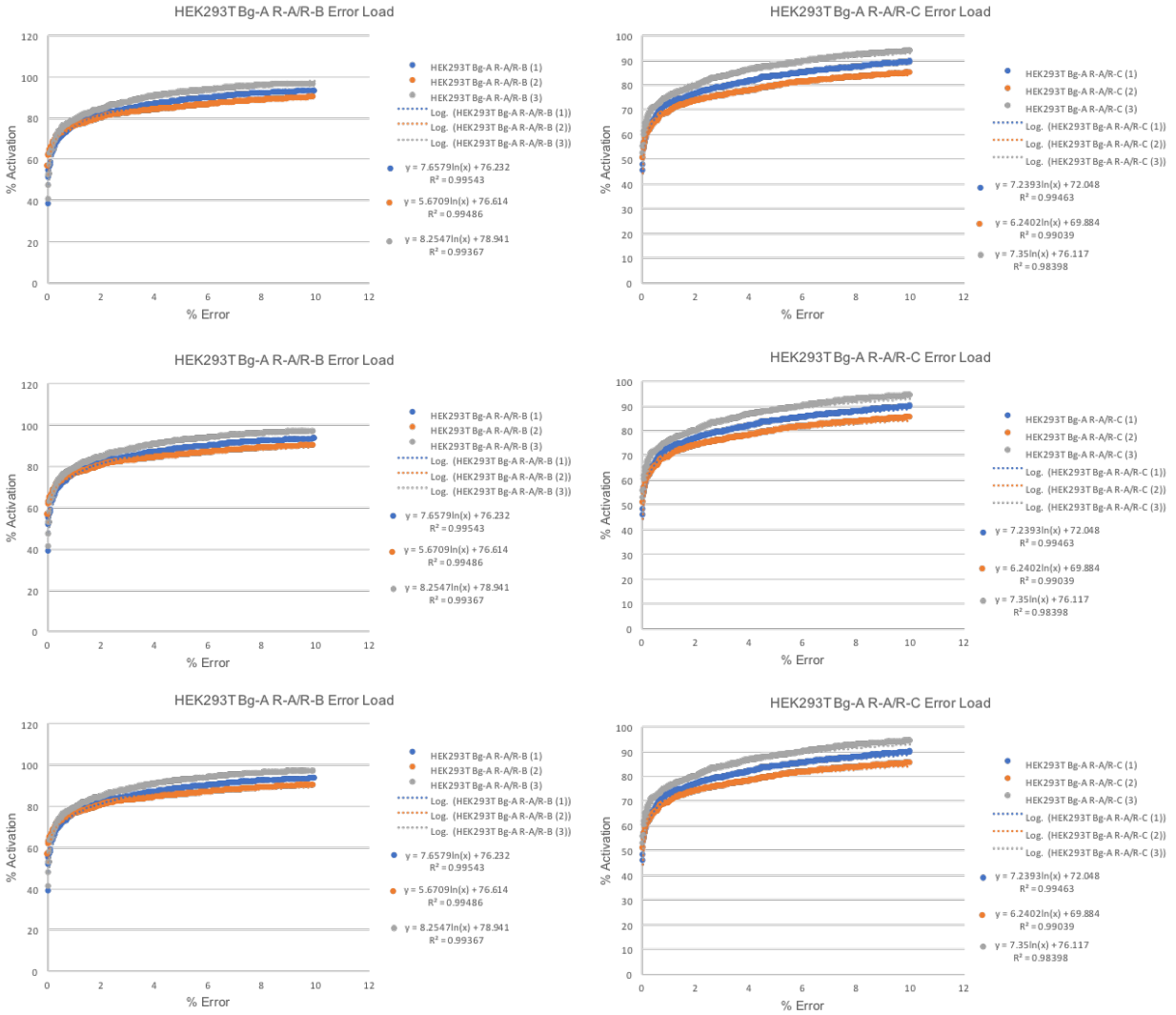




Supplementary Figure S3. Lineage specific gene activation efficiency with increase concentrations of dCas9-VPR in coordination with 1x, 3x, or 6x barcode landing pads. Time lapse fluorescent analysis of percent green object confluence of HEK293Ts Bg-A and Bg-B populations co-transfected with 0, 100, 300, and 900ng of dCas9-VPR and 80ng of Recall-A_GFP plasmids with a 1x, 3x, or 6x barcode array in a 24 well plate. These graphs compare recall activation efficiency of increasing amounts dCas9-VPR when co-transfected with 80ng Recall-A_GFP plasmids with a 1x, 3x, or 6x barcode array.

HEK293T Error Load

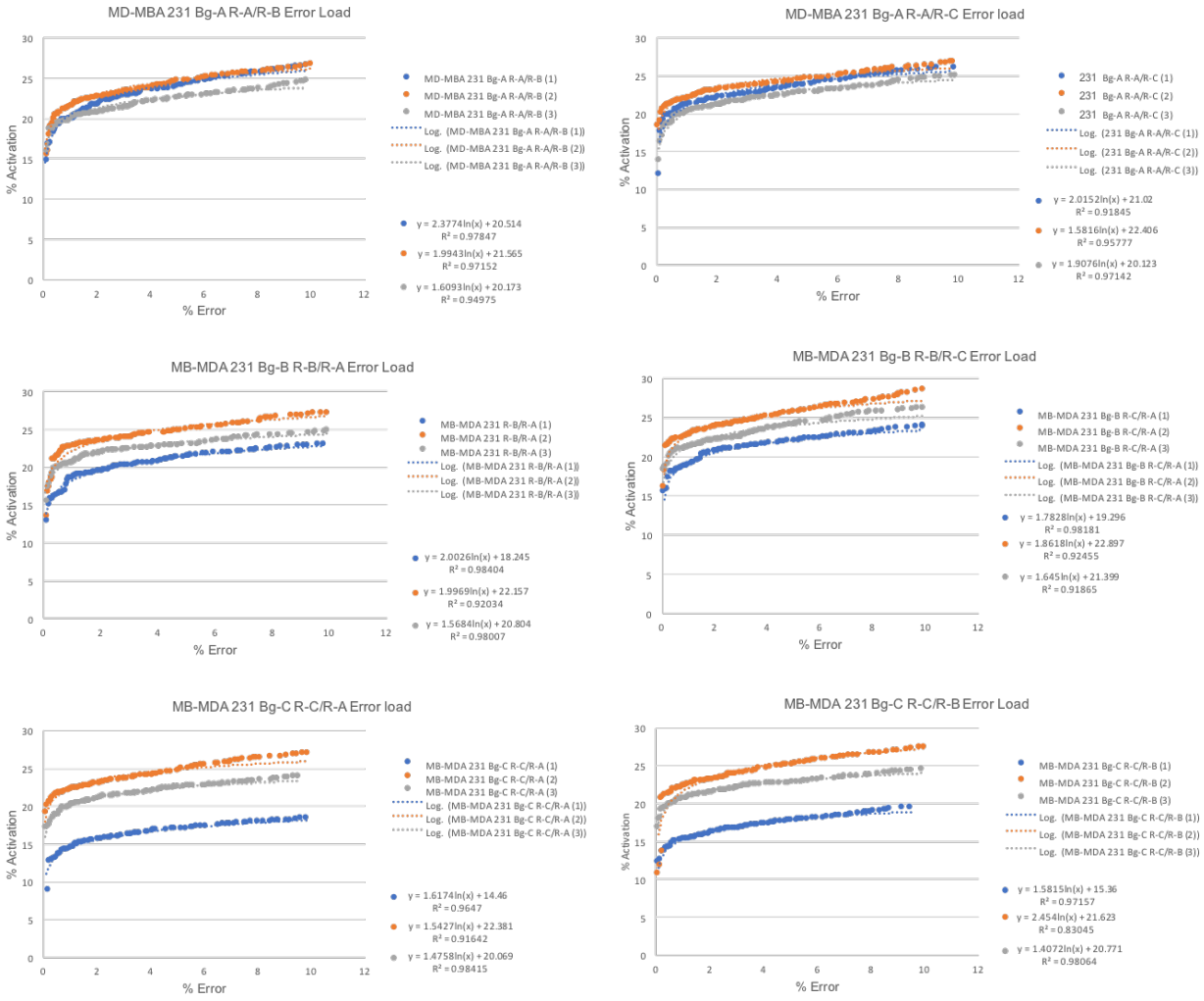
a



Supplementary Figure S4

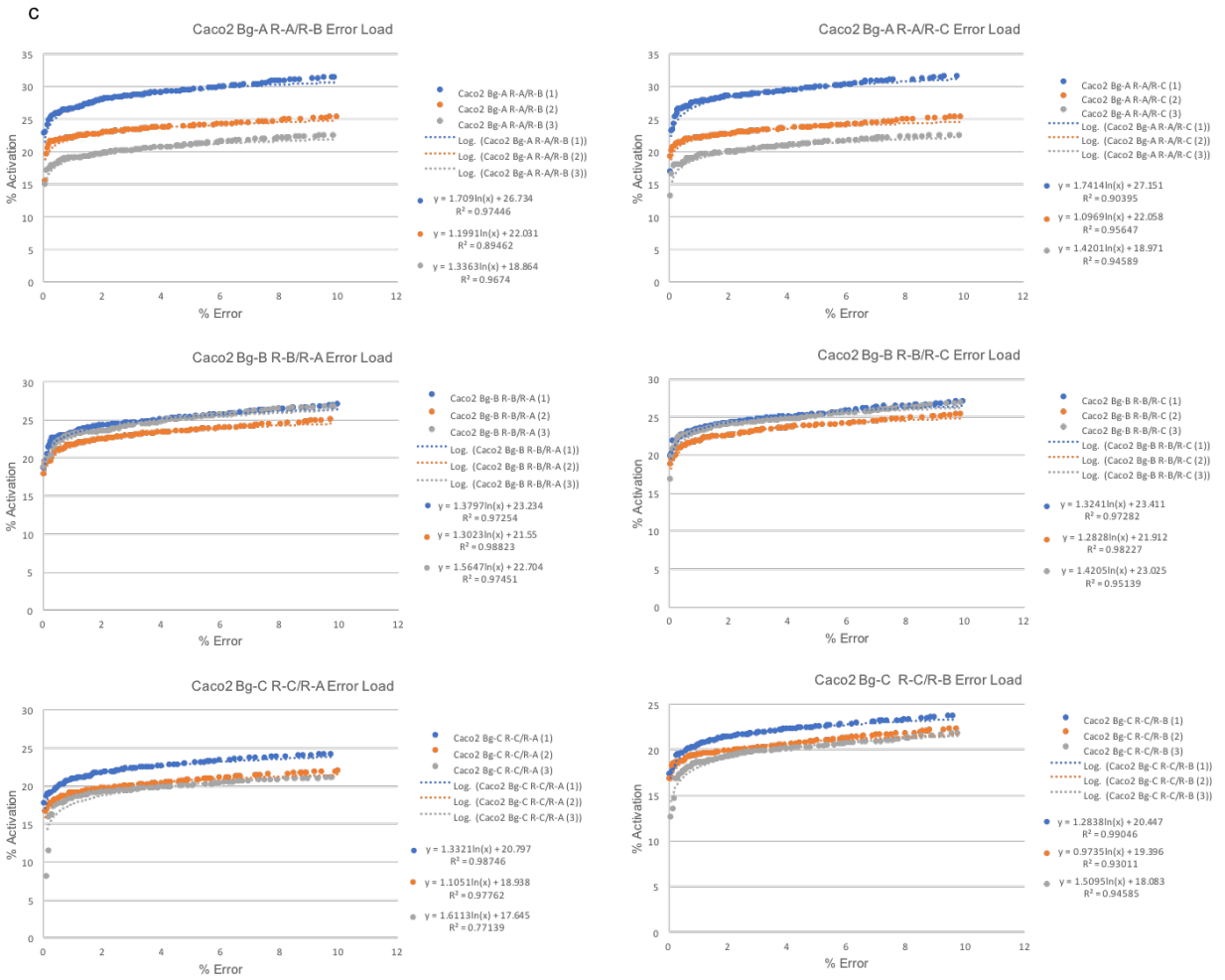
MB-MDA 231 Error Load

b



Supplementary Figure S4

Caco2 Error Load



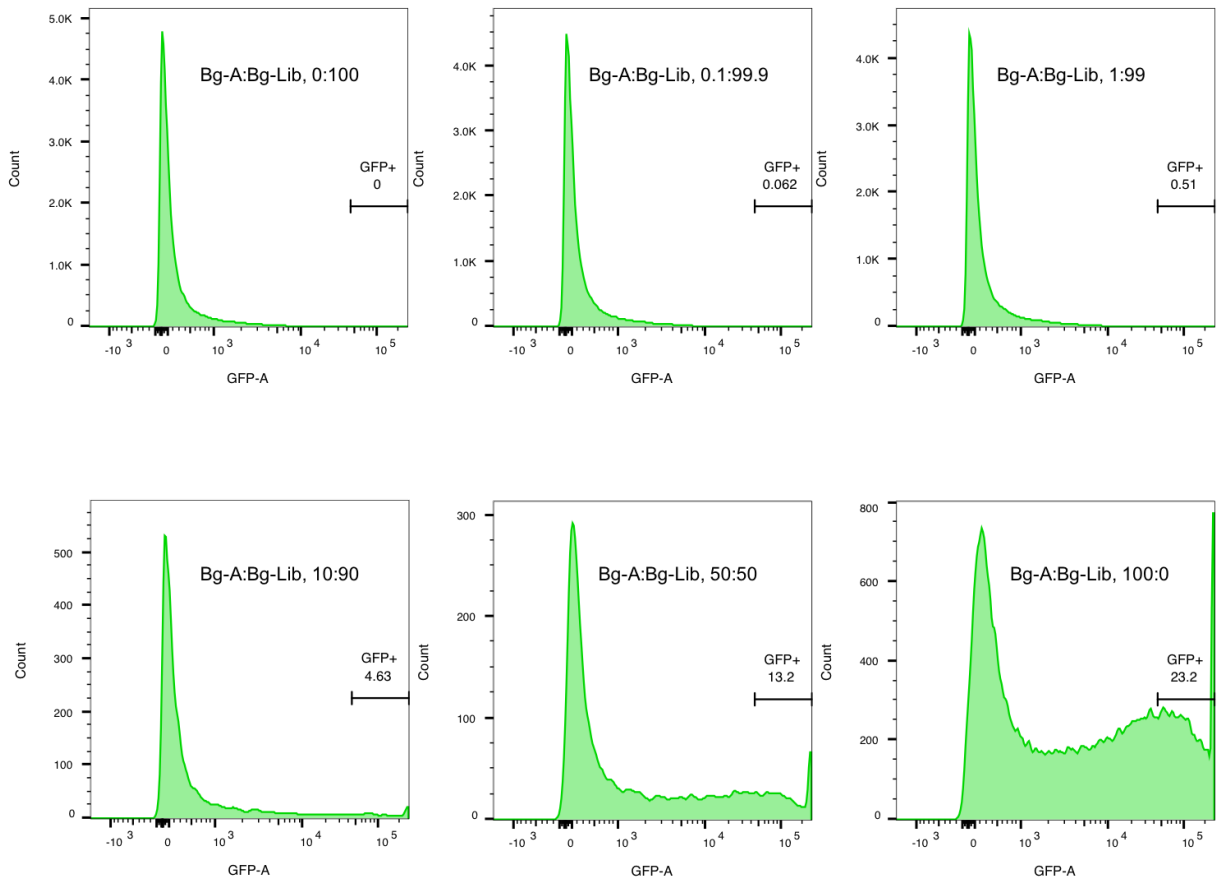
Supplementary Figure S4. Error load quantification.
Independent barcoded populations, Bg-A, Bg-B, and Bg-C, were generated in cell lines: HEK293T (a), MB-MDA 231 (b), and Caco2 (c). Barcoded populations were co-transfected with each of the Recall plasmids (R-A, R-B, R-C) containing GFP + dCas9-VPR plasmid independently, causing instances of either match or mismatch with regards to the barcoded gRNA and Recall plasmid. GFP expression was measured 48 hours after transfection. Error load was quantified by comparatively tallying the values of the GFP-histogram, from high to low GFP intensity, of matching and mismatching recall samples. Sum totals of matching recall events were tabulated with respect to each new accruing mismatch recall event. From these tabulations, % activation at a given error value was calculated as:

$$\% \text{ activation} = \frac{\Sigma\, matching\ recall\ events\ at\ given\ error}{total\ matching\ events\ analyzed}.$$

% error was calculated using the formula:

$$\% \text{ error} = \frac{\Sigma\, mismatching\ recall\ events}{\Sigma\, matching\ recall\ events}.$$

% activation and % error values were charted onto a scatter plot and, using least squares fitting, exponential equations were generated to model the data. (**Suppl Fig S4**). Fits for a vast majority of samples had an R^2 value of > 0.95. Experiments were performed in biological triplicate.



Supplementary Figure S5. HEK293T Bg-A/Bg-Library lineage dilution standard.
To initialize a standard and generate the gates for lineage isolation, a range of HEK293T Bg-A/ Bg-library dilutions were plated in a 6 well plate: 0% Bg-A, 0.1% Bg-A, 1% Bg-A, 10% Bg-A, 50 % Bg-A, and 100% Bg-A. The barcoded cell populations were co-transfected with Recall-A_GFP and dCas9-VPR. Cells were analyzed via flow cytometry 48 h post transfection. Sorting gates were set based off of the 0% Bg-A to maximize capture of GFP^+ cells while minimizing the capture of noisy non-lineages of interest.