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Supplemental information

An integrated pipeline

for mammalian genetic screening

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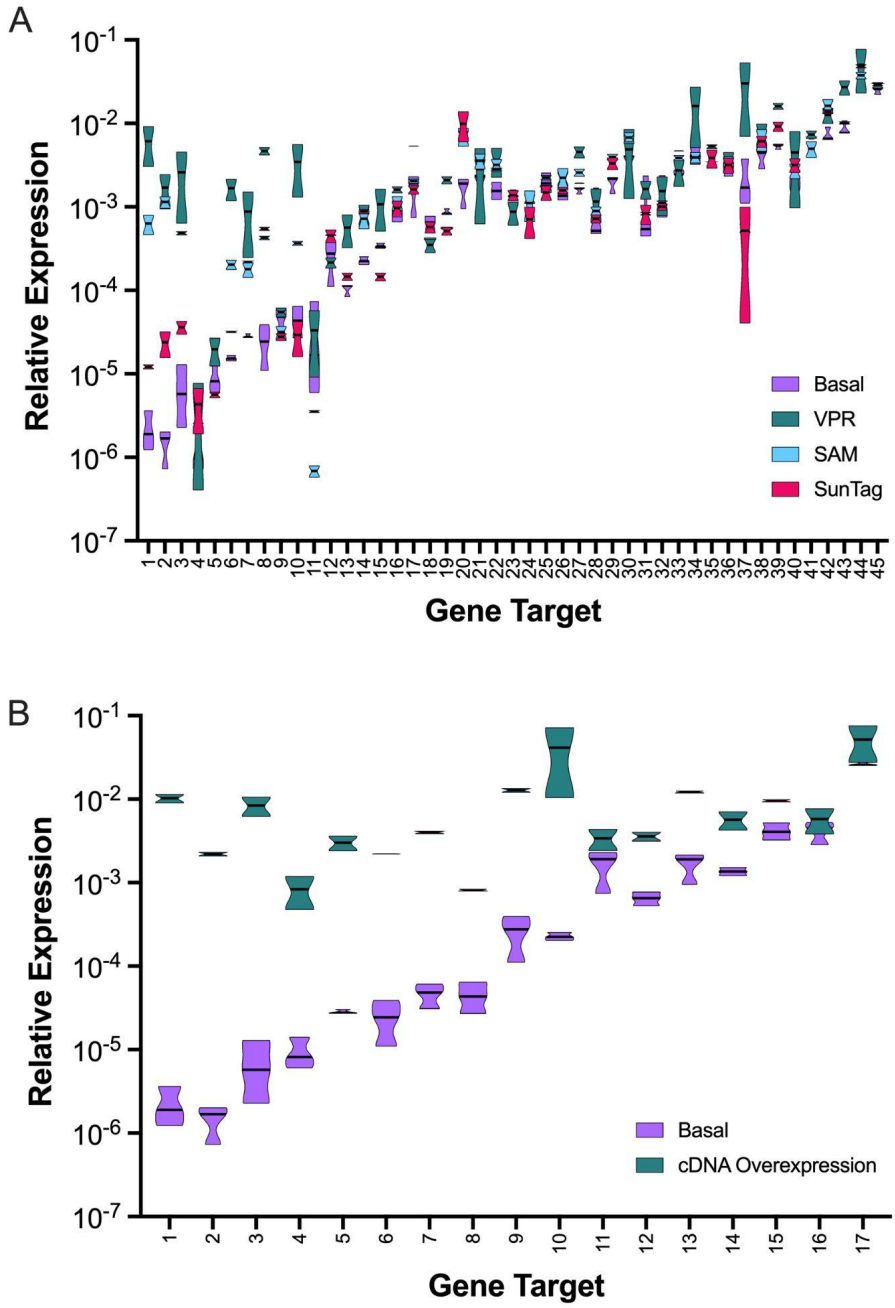
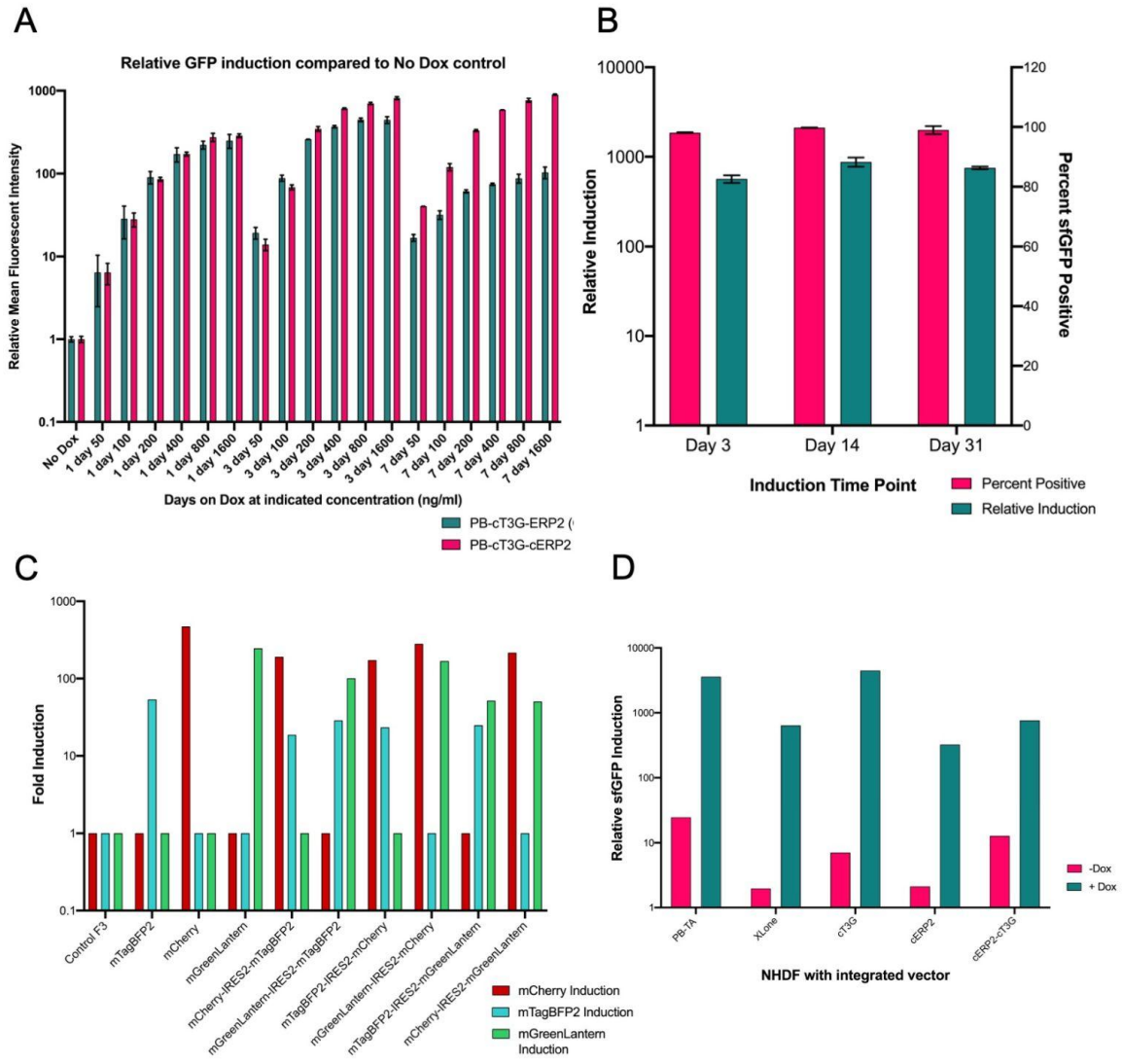
Supplementary Figures

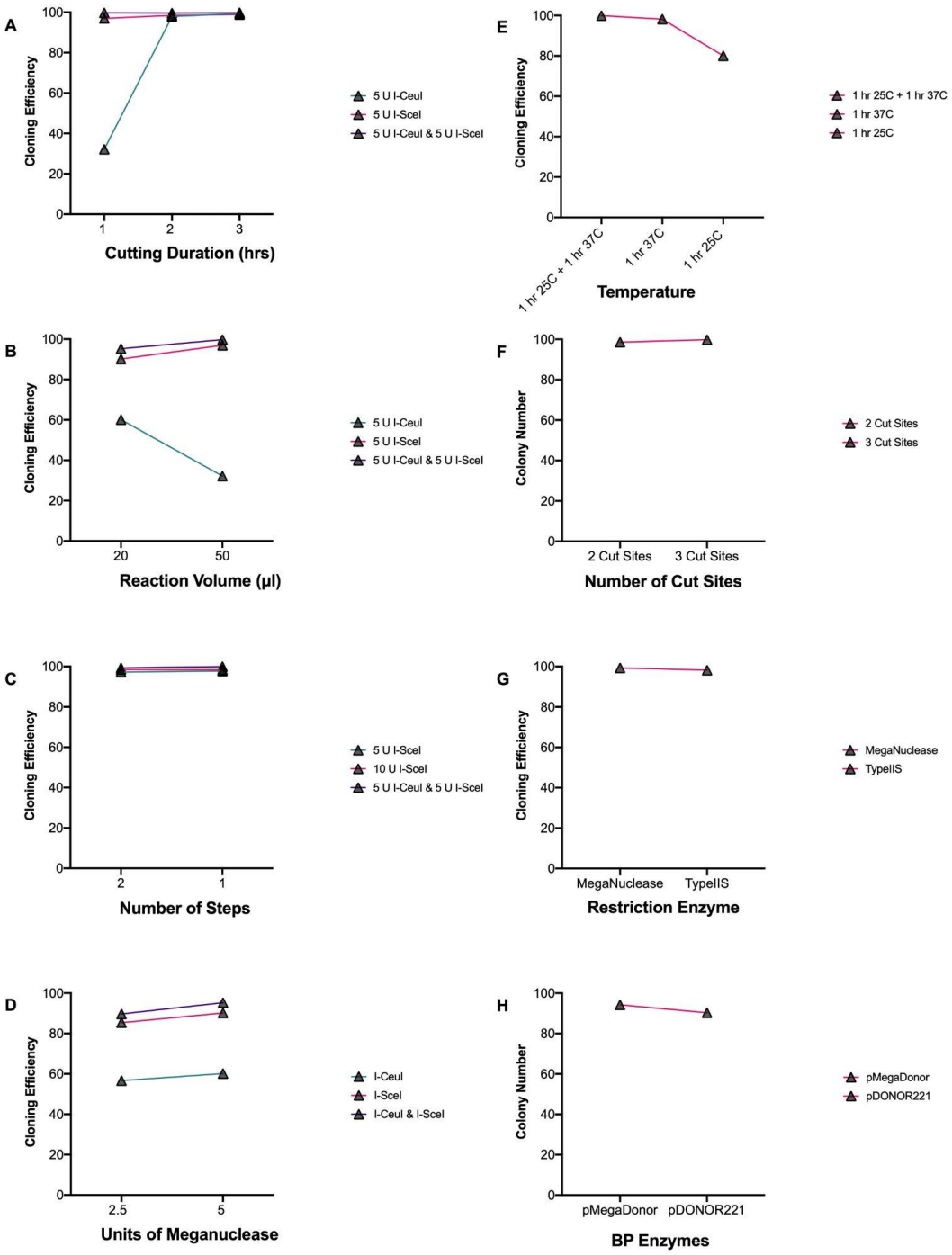
1. RT-qPCR from CRISPRa and cDNA tool Comparison
2. Flow cytometry of cDNA Vectors
3. Optimized reaction conditions for MegaGate cloning
4. Copy number assessment in single cell and bulk populations
5. Targeted RNA sequencing optimization (TAR-Seq)



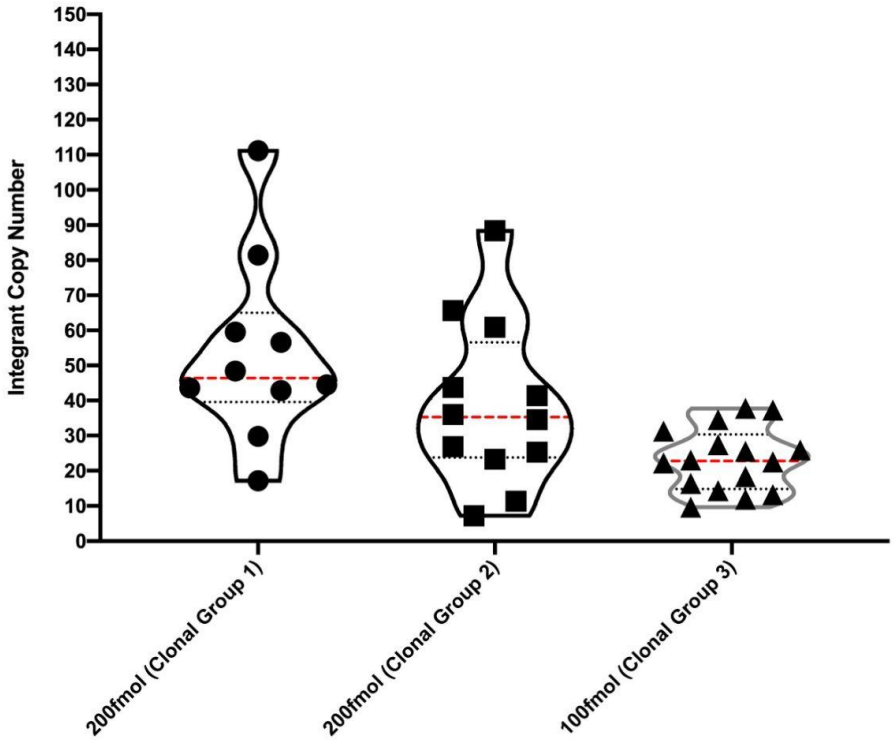
Supplementary Figure 1. Relative expression of gene targets, Related to Figure 3. RT-qPCR was performed to determine the expression for 45 genes (A) or 17 genes (B) after 48 hour induction via CRISPRa (A) or cDNA (B) in biological duplicates. Relative expression is calculated using deldelCq method relative to GAPDH. The basal gene expression level is calculated from a no induction vector control condition.



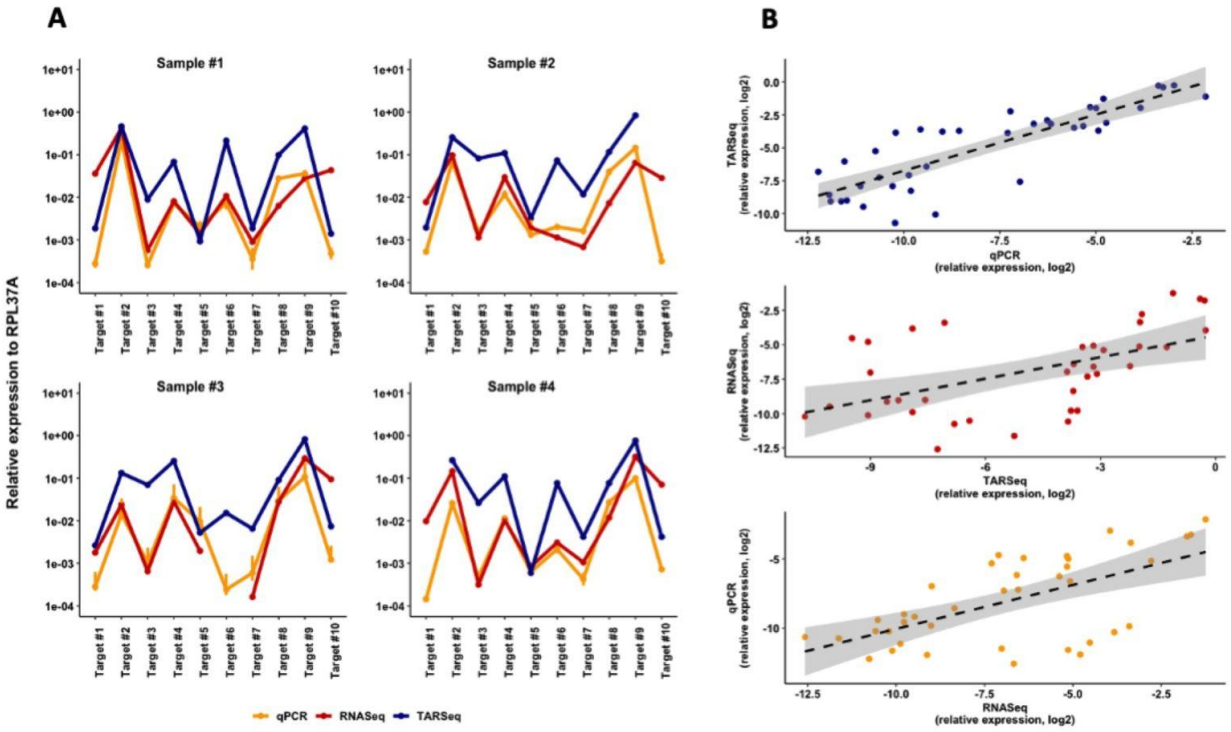
Supplementary Figure 2. cDNA expression vector dynamics were compared using flow cytometry, Related to Figure 3. Relative expression was calculated as the relative mean fluorescent intensity of the fluorescent construct compared to a basal no induction condition. A) Expression dynamics under dox titration at three time points B) Stable vector expression over one month in hiPSCs. C) dual expression of IRES2-Fluorescent proteins D) Expression comparison of five vectors in NHDFs after integration and selection



Supplementary Figure 3. Optimization reactions for MegaGate Cloning, Related to STAR Methods. Cloning efficiency is determined as the number of colonies on the plus insert plate compared to the total number of colonies.



Supplementary Figure 4. Copy number in single cell isolated and expanded clones for three different gene sets and input ratios, Related to Figure 4. Copy number was determined via RT-qPCR via the 2*delCq method compared to the single copy number gene RPP30.



Supplementary Figure 5. Validation of Targeted RNA-Sequencing (TAR-Seq) with custom gene panels, Related to STAR Methods. A) Relative expression with respect to RPL37A of a target gene panel in 4 different NHDF samples across 10 different targets as quantified by TAR-Seq, RNA-Seq and RT-qPCR. Target 10 in Sample #2 was not identified due to low abundance. B) Gene expression correlation of 10 genes in 4 different samples between TAR-Seq, RT-qPCR and RNA-Seq.