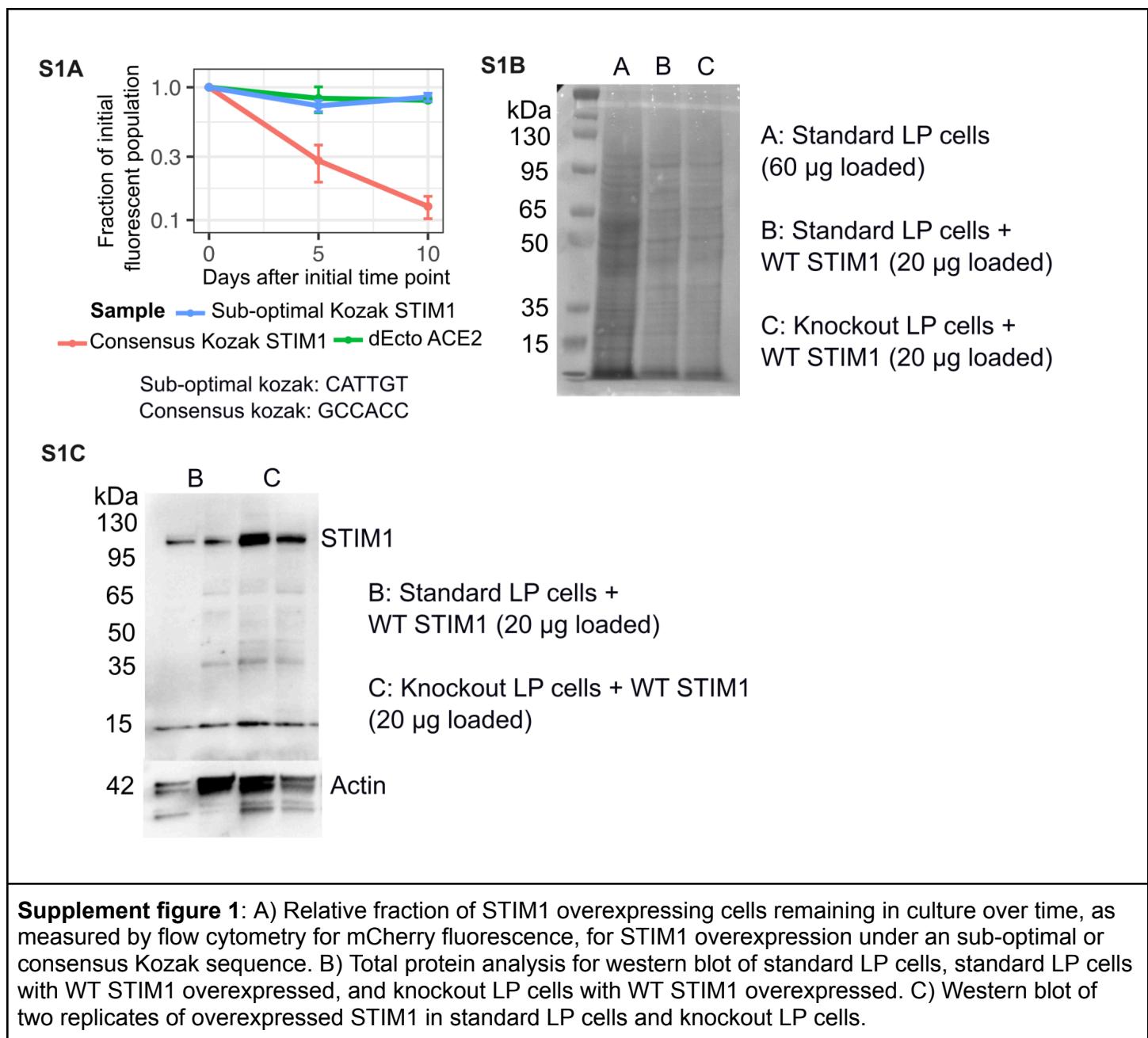


Additional files

Supplement Tables:

1. Supplement Table 1: Survival scores for cell survival DMS in standard LP, knockout LP, bacteria strains, and validation experiments
2. Supplement Table 2: Primers used for NGS experiments
3. Supplement Table 3: Names of FASTQ files
4. Supplement Table 4: qPCR primers used for gene expression experiments
5. Supplement Table 5: Compilation of results from all databases accessed for this study

Supplement Figures:



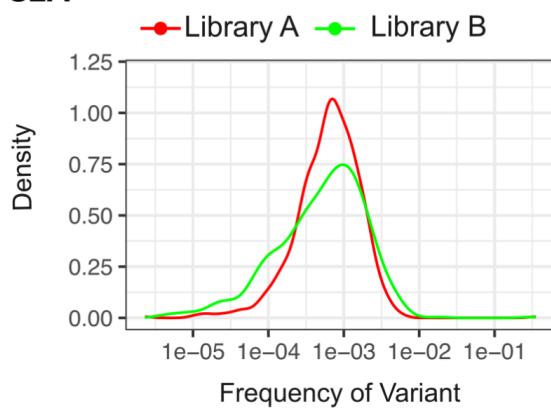
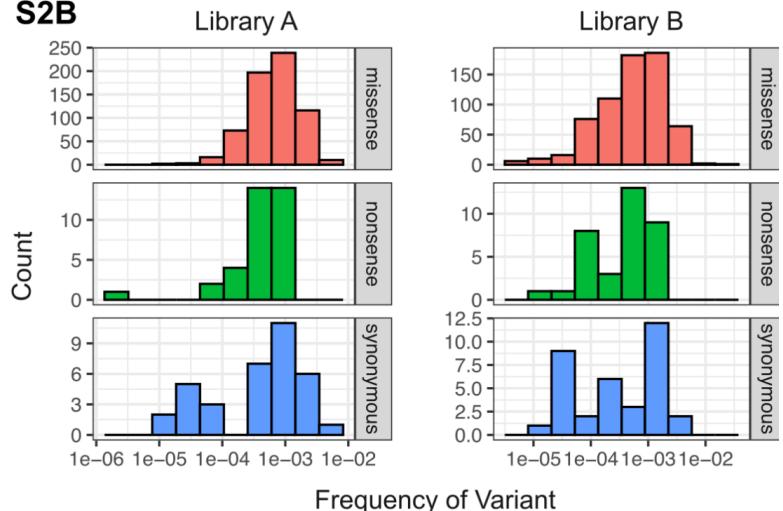
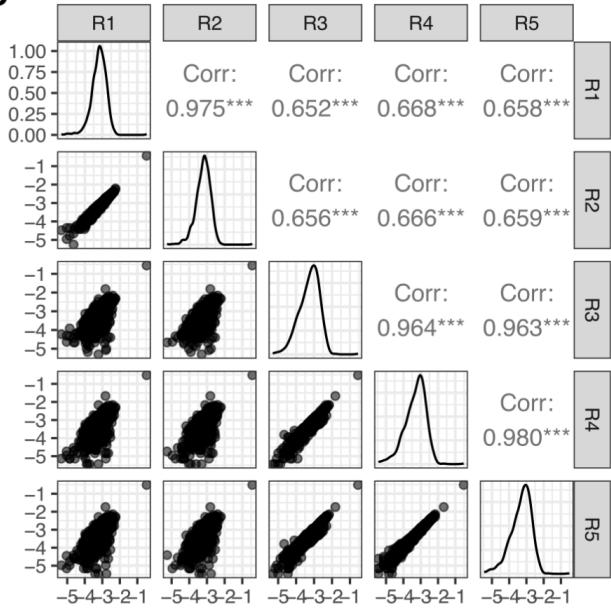
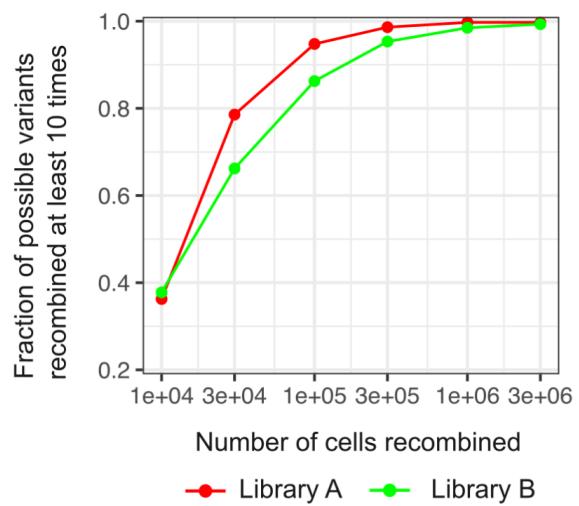
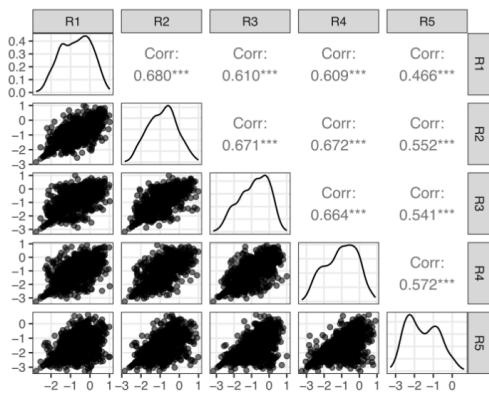
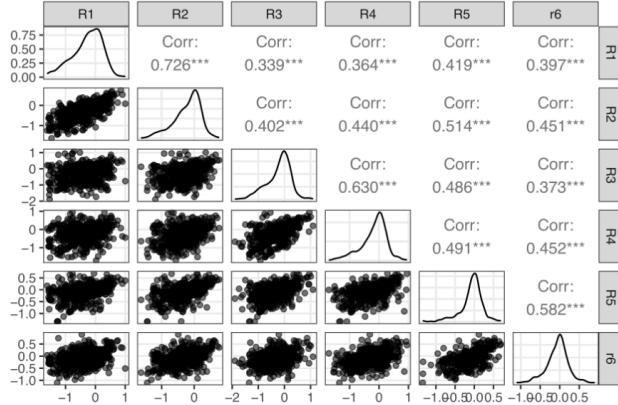
S2A**S2B****S2C****S2D**

Figure supplement 2: A) Density plot of frequency of variants in plasmid libraries used for standard (library A) and knockout LP (library B) DMS experiments. B) Histogram of missense, nonsense and synonymous STIM1 cEF-hand variants present in both plasmid libraries. C) Correlation plot of each sequenced replicate of the plasmid library. R1 and R2 were library A, R3-R5 were library B. D) Simulation to determine the number of recombined cells needed to observe a variant at least 10 times.

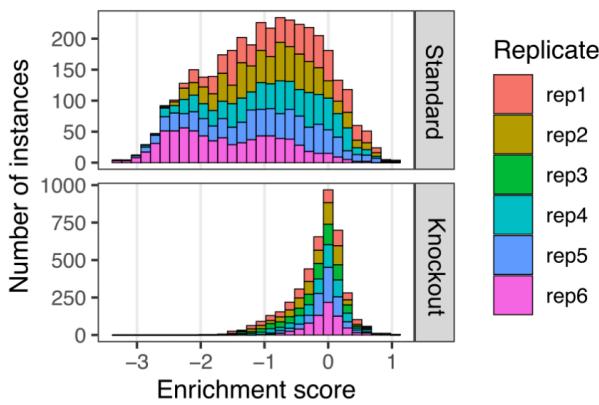
S3A



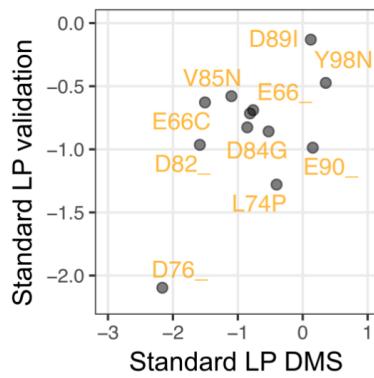
S3B



S3C



S3D



S3E

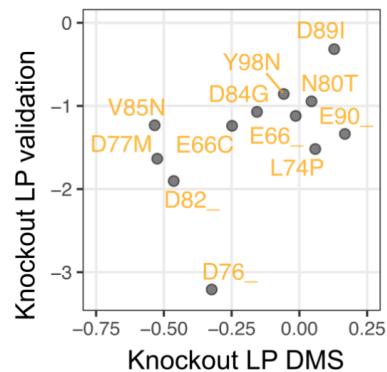


Figure supplement 3: A) Correlation plot of standard LP DMS replicates. B) Correlation plot of knockout LP DMS replicates. C) Histogram of variant score distributions seen in standard and knockout LP DMS, colored by replicate experiment. D) Scatterplot of DMS score vs small scale validation scores in standard LP cells. E) Scatterplot of DMS score vs small scale validation scores in knockout LP cells.

S4A

Possible -35 sequence
TTGTAA

Cryptic Pribnow box
TGTAAT

ACGACGTTGTAAAACGACGCCAGTGAATTGTAATACGACTCACTATAGGG
TAATACGACTCACTATAGGG

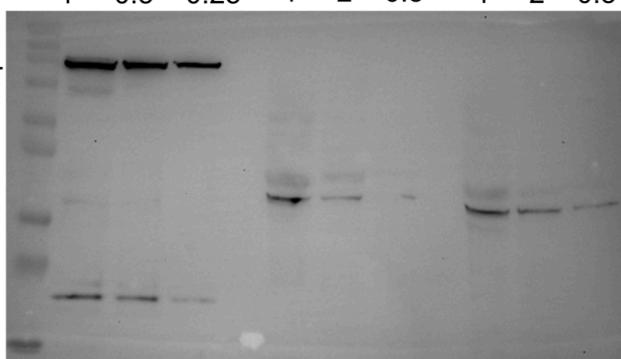
T7 promoter

23 nucleotides 5' of a partial Shine-Delgarno sequence (TCGAGGT), and 35 nucleotides 5' of an ATG initiating methionine in-frame with the STIM1 open reading frame.

S4B

lysate conc (μ g/ μ L)	STIM1-mGL in standard LP cells			attB-mCherry DH10 β			attB-STIM1 DH10 β		
	1	0.5	0.25	4	2	0.5	4	2	0.5

STIM1-mGL



Total protein (Ponceau S stain)

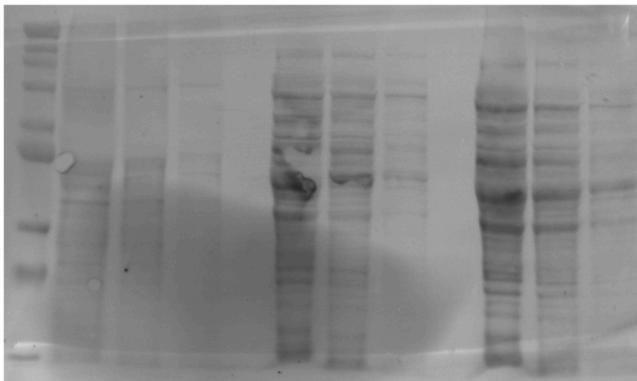
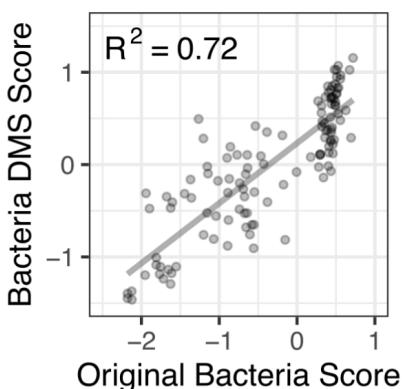
**S4C**

Figure supplement 4: A) Schematic representation of the cryptic bacterial promoter retrospectively identified in the plasmid. B) Western blot of STIM1 overexpression in DH10 β bacteria. C) Scatterplot of transformation scores from first DMS to the scores from the second DMS in DH10 β cells.

S5A

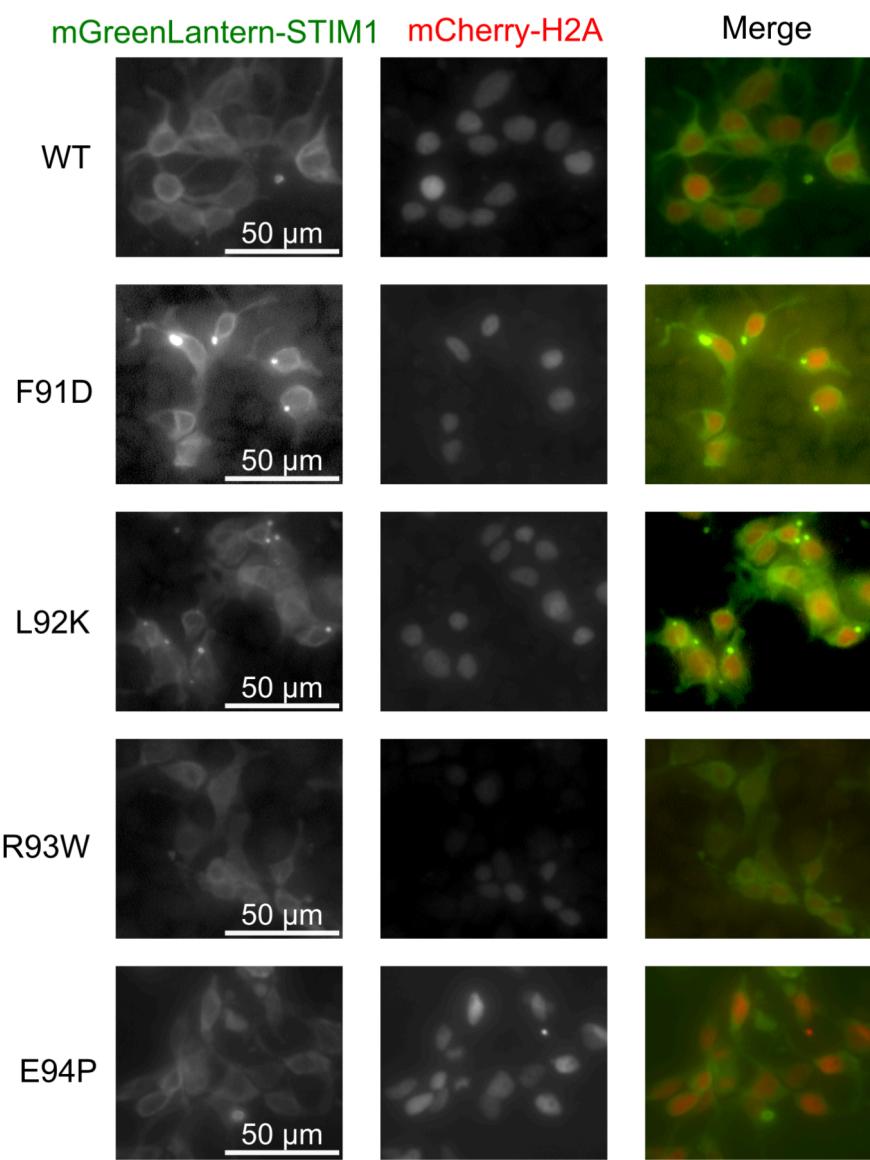


Figure supplement 5: A) Raw fluorescence microscopy images of mGreenLantern-tagged STIM1 variants in standard LP cells.

S6A

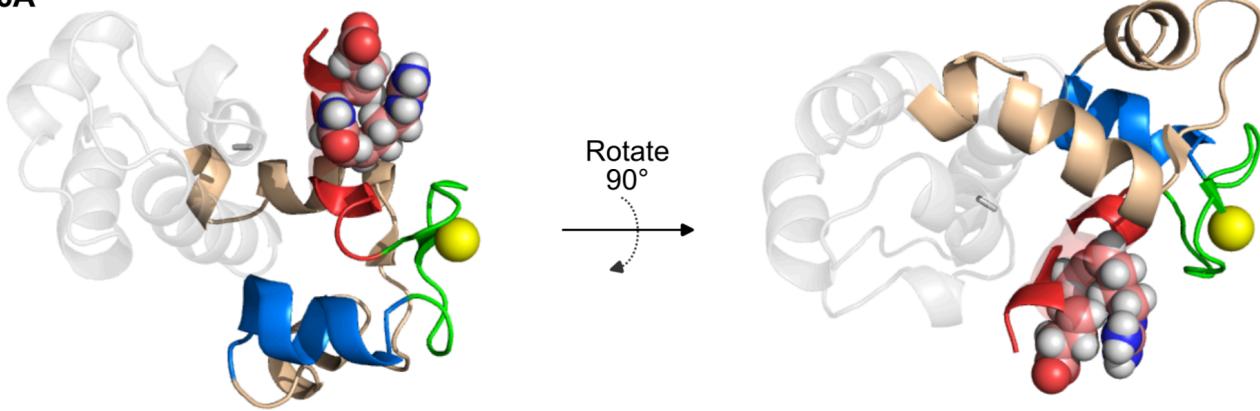


Figure supplement 6: Pymol visualization of the STIM1 EF-SAM calcium loaded NMR structure (PDB: 2k60), with the STIM1 cEF-hand positions differing in mammalian orthologs (Glu66, Arg69, Asn70) shown as spheres. The calcium ion is shown as a yellow sphere. Helix α 1 is color red, the EF-motif is colored green, and helix α 2 is colored blue. The hidden EF-hand is colored beige, whereas the SAM domain is shown in semi-transparent gray.

