

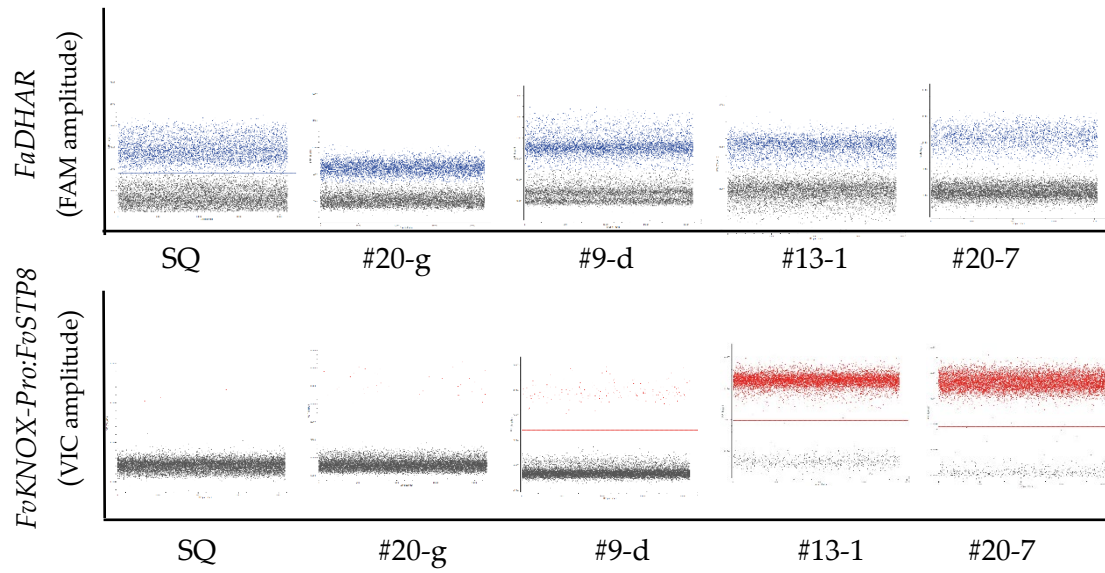
**Supplementary Table S1** List of primers used for vector construction and PCR analysis of transformants.

Primer name	Sequence (5'-3')	Amplicon size (bp)	purpose
CM2268	gtacggtcagtccttATGGCCGAGGTTT CTCG	1590+32	FvSTP8 CDS with adaptor (lowercase letters)
CM2269	caggagagttgtgatCTACAACTGTGTAA CAGGATC		
ZT067	aagggactgaccgtacccggggatcgat		Reverse PCR for
ZT068	atcaacaactctcctggcgac	11772	vector backbone 1
CM2328	gttacacagtttagATTTTGAAGAAGCGT CAGGGTTC	401+30	FvSTP8 terminator with adaptor (lowercase letters)
CM2329	cgaattaattcggggCACAACAAGCACAA AACTTACA		
CM2269	caggagagttgtgatCTACAACTGTGTAA CAGGATC	10748	Reverse PCR for vector backbone 2
ZT069	ccccgaattaattcggcgtaattcag		
CM2140	gtgatcgattccgagcaaac (3' end of promoter)	528	Direct PCR identification of transformants
CM2340	AGCAAGATCTTGAGCTGCAG (mid FvSTP8)		
pMDC162-Kan1	caatgtcataccactgtccgc		PCR analysis the
pMDC162-Kan2	ctcatgagtgaggccgatg	400	potential integration of backbone DNA

**Supplementary Table S2** Sequence information of the oligonucleotides used for droplet digital PCR (ddPCR) in this study.

Target	Oligo Name	Sequence (5'-3')	Amplicon (bp)	Note
Reference gene (glutathione S-transferase DHAR)	Ref-F	AGGCACATGTATGTGTTC TCTCA	178	Oligos used match one copy per genome: FxaC_26g17540
	Ref-R	GTACAGTTTTGGTGCCAA		
	Ref-Probe	GC		
		FAM- CAGTGACTTTCTCTCCAGC AATGT		
T-DNA region of pMF-P03606:FvS TP8	Intragenic	GTGATCGATTCCGAGCAA	147	Oligos match the junction region of P03606 (intragenic-F) and FvSTP8 CDS (Intragenic-R) on the intragenic construct.
	-F	AC		
	Intragenic	GAGAAACCTCCGGCCATA		
	-R	AG		
	Intragenic -Probe	VIC- CGAATTCGACCCAGCTTT CTTGTA		

**Figure S1.** One-dimensional plot of droplets measured for fluorescence signal emitted from the endogenous reference gene *FaDHAR* (FAM<sup>TM</sup> labeled, positive droplets are blue) or the intragene *FvKNOX Pro:FvSTP8* (VIC<sup>TM</sup> labeled; positive droplets are red) in four distinct transformants (#) and untransformed cv. Shanghai Angel (SQ). Negative droplets are black.



**Figure S2.** The droplets visualized in two dimensions for ddPCR analysis with the genomic DNA templates of SQ (untransformed Shanghai Angel), #20-g, #9-d and #20-7. The colors are as described in Fig.S1 but the droplets with both fluorescent probes are purple.

