

Supplementary Figure 1 – **Preliminary testings of the dexamethasone-inducible system using a few selected pOpOff-SAL1hpRNAi lines in Arabidopsis.** A) Leaves of T1 transformants were either painted with no dex or with 20 μM dex before GUS staining was checked 2 days after treatment (DAT) on whole leaf or 5 hours after treatment (HAT) on partial leaf painting. B) *SAL1* transcript quantification from leaf tissues of three-week-old T3 plants after one week of 20μM dex treatment via soil-drenching. n=3 and error bars indicate standard deviation. Significant differences (ANOVA, p<0.05) are denoted by a, b, c. C) Total protein was extracted from leaves of four-week-old T3 plants treated with or without dex via soil drenching every three days since germination (n=2), SAL1 and PEPC as loading control were probed. Dilution series of 1:1, 1:2 and 1:5 were loaded for each sample.





Genotype/line

51 13

Supplementary Figure 2 – Screening transgenic lines to identify the potential of SAL1 silencing efficiency of pOpOff system. A) Leaves of four-week-old T2 plants were harvested for GUS staining two days after painted with $20\mu M$ dex. Three-week-old seedlings germinated and grown on MS supplemented with 20µM dex were harvested for B) SAL1 transcript quantification relative to the wild-type Col-0 control via qRT-PCR [n= 3 technical replicates, error bars = standard deviation, significant differences = ANOVA, post-hoc test relative to Col-0: *** denotes p<0.001, ** denotes p<0.01 and * denotes p<0.05] and C) PAP quantification using HPLC. At least 10 seedlings per transgenic line were pooled for each quantification [n=3 biological replicates for Col-0 and sal1-6, error bars = standard deviation]. Student T-Test relative to Col-0 control was performed; *** denotes p-value<0.001.

Col-O 0.6

[PAP] (pmol / mg FW)

c)

0.4 0.2 0





Supplementary Figure 3 – Characterization of T2 35S:*SAL1***hpRNAi lines.** A) Representative photos of selected lines at 3.5 weeks old are shown. B) Selected 35S:*SAL1***hpRNAi lines**, particularly those with "higher-than-Col-O" PAP accumulation at T1 generation, were re-tested for PAP levels at T2 generation, where five seedlings per line were pooled.



Supplementary Figure 4 – Representative images of T1 35S:*SAL1***amiRNA lines at four weeks old.** A) 35S:*SAL1***ami339.** B) 35S:*SAL1***ami1002.** Each pot represents one independent transgenic line.

a)



Supplementary Figure 5 – Summary findings of SAL1 silencing strategies using: dex-inducible SAL1hpRNAi (blue), strong constitutive hpRNAi (yellow) and amiRNA (green). A) Boxplot of SAL1 transcript levels relative to wild-type Col-0. B) Boxplot of PAP levels.



Supplementary Figure 6 – Silencing of *PDS* in Col-0 and *sal1* transformed with 35S:*PDS*hpRNAi.

Vector	Empty Vector						pOpONSAL1					
Dex treatment on	No		Yes			No			Yes			
germination												
Dex treatment duration	0	1	2	0	1	2	0	1	2	0	1	2
on soil (weeks)												
Abbreviation	EN0	EN1	EN2	EY0	EY1	EY2	PN0	PN1	PN2	PY0	PY1	PY2



Supplementary Figure 7 – Average rosette area of three independent transgenic lines of empty vector control and pOpON-SAL1 at the end of the different dex treatment regimes. Average rosette area of 3 to 4 plants per line at 38 days old was quantified using LemnaTec Scanalyzer and the error bars represent standard deviation. The abbreviated label on the x-axis is defined in the table above the bar graph.



Supplementary Figure 8 – Rosette area, plant images and PAP quantification of individual T1 early developmental stage specific complementation of *sal1* transgenics together with Col-0 and *sal1*-6.



Supplementary Figure 9 – Rosette area and PAP levels of T2 early developmental stage specific complementation of *sal1* transgenics in comparison to Col-0 and no pro:*SAL1* controls. $N \ge 5$.



Supplementary Figure 10 – Days of survival during drought and their corresponding rosette area of T2 individuals of ABI3:*SAL1*, TZF6:*SAL1* and LEC1:*SAL1* plotted in comparison to the predicted upper and lower limits of Col-0 control. Different colours denote different independent lines.

1	GACATATATT	TATCTTCTTG	AAAAGCGA <mark>AT</mark>	GATGTCTATA	AATTGTTTTC	
51	GAACAGCGAA	GGCTCCGCTT	CAATCATTTG	TAGCAGTAAG	AACGAATTCG	
101	AGACCTAGAA	ATTCATCGAA	CCGTCTCGTT	TCTGTATTCG	GACGCAAGTC	
151	TTCTTCTCCT	TCATTTGTTA	CTCTCAGAGT	TGTTTCATCG	ATGGCTTACG	
201	AGAAAGAGCT	TGATGCTGCT	AAGAAAGCTG	CTTCACTCGC	TGCTCGTCTC	
251	TG TCAGGTTA	GGGTTTTTTC	GATTCAATCA	TGACCCATAG	ATTCTAAAGT	
301	TTGATTCTTT	AAGAAACCCA	TTTTGTAAAT	CTTCCAAATT	TCGTTTAACA	
351	TTTTGTGTTT	ATTGTGCATT	GCATCTGTAA	TTGGGAATAG	ATTCTAGTGA	
401	TATAGTGTAA	TGGTCCTCTA	CATACGAAGC	TCGTGTAAAT	CTTTGATCAA	
451	AATCTTATCT	TTGTGTTTTG	GGTTTGTT	AGAAAGTTCA	AAAGGCTTTG	
501	TTGCAATCAG	ATGTGCAATC	AAAATCTGAT	AAAAGTCCAG	TGACCGTTGC	
551	TGATTATGGT	TAGTTTGTTA	TACCTGTCCC	TGATTAGAAA	AAGCTCTTCT	
601	CTTTGAATGT	TACTGAGATT	GTTAGGAAAT	CACTTAATTT	GATCTGTCTT	
651	GTGTTGAATT	TCA <mark>GGTTCAC</mark>	AGCAGTTGT	TAGTTTAGTC	TTAGAAAAAG	amiRNA339 target region
701	AGCTCAGTTC	TGAACCCTTT	TCATTGGTGG	CTGA <mark>AG</mark> AGGT	GAAACTGCTT	
751	AATAAATCCT	TGTTAGATGT	CTCACACTTT	ACTTATCTTT	GAGTTTGTGT	
801	TTATGGACTC	ACATTGTCTA	AAATGATCTA	TAT <mark>AGGACTC</mark>	AGGCGATCTA	
851	CGCAAGGATG	GTTCTCAGGA	TACTCTGGAG	CGCATCACAA	AACTCGTGAA	Region targeted by SAL1hpRNAi
901	CGACACTTTG	GCTACCGAGG	AATCGTTTAA	TGGCTCTACT	TTGTCTACTG	
951	ATGATCTACT	TAGAGCCATT	GAC <mark>TGT</mark> GGAA	CATCTGAAGG	TGGTCCAAAT	SAL1 gRT-PCR primers targeting region
1001	GGTCGACACT	GGGTCTTGGA	TCCAATTGAT	GGCACTAAAG	GGTACGTTTT	
1051	AAAACTAACT	AGCCTAAAGT	CAAATCTTCT	TATTTCAGAG	AAAATGTAAA	
1101	TTTGATAGAA	TGTTGAGTCA	GATGTTATGT	TCCTGACACT	GAGCATTTTC	
1151	ATGATTTTA <mark>G</mark>	ATTTCTGAGG	GGAGATCAAT	ACGCAGTAGC	ACTAGGATTG	
1201	CTCGAGGAAG	GGAAAGTAGT	TTTAGGTGTG	CTTGCTTGTC	CAAACTTGCC	
1251	GTTAGCATCC	ATAGCAGGAA	ACAACAAGAA	CAAATCTTCG	TCAGACGAAA	
1301	TTGGA <mark>TGC</mark> CT	CTTCTTTGCT	ACAATTGGTT	CAGGGACATA	TATGCAGCTC	
1351	CTAGATTCAA	AATCTTCTCC	TGTAAAAGTG	CAAGTCTCTA	GTGTTGAGAA	
1401	TCCTGAAGAG	GCATCGTTCT	TCGAGTCATT	CGAAGGAGCT	CACTCTCTAC	
1451	ATGACTTATC	CAGCTCCATT	GCCAAT GTAA	ATTGCTTCTT	TCCTTCCATG	
1501	TGATTCCAGC	TAATAGCTAA	CTAATTTTCC	TCATCCATTT	GATCATGTTC	
1551	TATGTTGTAA	TATACAG <mark>AAA</mark>	CTCGGTGTCA	AAGCTCCACC	AGTCCGTATT	
1601	GATAGCCAAG	CAAAGTATGG	AGCTTTATCA	AGAGGAGATG	GAGCTATATA	amiRNA1002 target region
1651	CTTACGGTTT	CCTCATAAAG	GATACCGCGA	AAAGATTTGG	GACCATGTCG	
1701	CTGGTGCTAT	AGTTGTTACA	GGTAACATTA	AAGCTTACTC	TCTATGAAGC	
1751	TAATTTTATA	GTGTCGACAT	GCGGATGTAA	ATAGATAAGG	AATGCAAGGT	
1801	TGATTCTTCT	TTTTGGTG <mark>CA</mark>	GAGGCGGGTG	GAATAGTGAC	AGATGCAGCA	
1851	GGAAAGCCAC	TGGATTTCTC	GAAAGGGAAG	TATCTTGATT	TGGACACAGG	
1901	CATTATCGTT	GCTAACGAGA	AGCTAATGCC	TCTGCTTTTG	AAAGCAGTTC	
1951	GTGACTCCAT	AGCTGAGCAA	GAGAAAGCTT	CAGCTCTCTG	ATTTGTTTTT	
2001	TTCTCTCGTA	CGTTCTTTGT	TTCTCTGTAA	CTGTTGTTTC	ATTTTCTTTC	
2051	ACCGAATTTC	ACCAGTGAGA	ATTTCTTCCA	TTTTCGAAAA	AGAAATAAAA	
2101	ATGAAATTCT	GTTTTGGGCT	AA			

Supplementary Figure 11 - SAL1 genomic sequence. Exons are highlighted in yellow. Region (excluding introns) indicated in black bracket is targeted by SAL1hpRNAi; blue box is targeted by 5'-targetting SAL1amiRNA; red box is targeted by 3'-targetting SAL1amiRNA; green bracket denotes region amplified during qRT-PCR for SAL1 transcript quantification.



Supplementary Figure 12 – Calibration curve for the conversion of rosette area in pixel format from LemnaTec Scanlyzer software to area in cm². Green colour cards were cut into duplicated squares of specific dimensions (1cm X 1cm, 2cm X 2cm, up to 5cm X 5cm) and their images were captured and analysed using the Scanalyzer. These corresponding squares of known area were then cut into a series of interconnected pieces resembling an Arabidopsis rosette, or into disconnected pieces before images of them are captured and analysed again. This is to ensure the capacity of the image analysis to accurately detect and measure rosette area of Arabidopsis plants of varying size and rosette morphology.